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The British Mycological Society

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TRANSACTIONS

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Volume 44

Edited by
S. D. GARRETT and GRACE M. WATERHOUSE

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1961

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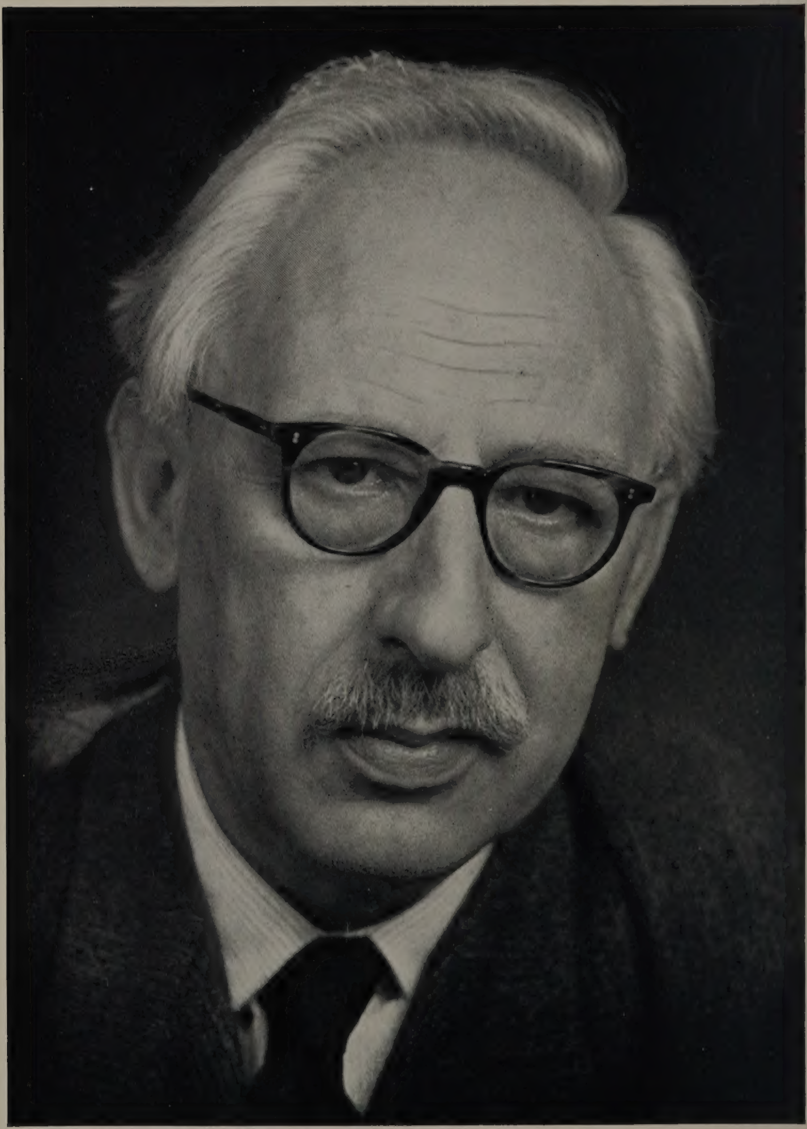

F. C. LARGE
President, 1960

Vol. 44, Part 1 March 1961

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PRESIDENTIAL ADDRESS

PURSUITS OF MYCOLOGY

By E. C. LARGE

(With Portrait and 9 Text-figures)

> '*To sport with Amaryllis in the shade,*
> *Or with the tangles of Neaera's hair.*'
> John Milton.

You have come, perhaps rightly, to expect from each year's President an account of some solid piece of mycological work that he has done for the advancement of our science. In this I am going to disappoint you. Although over the past 20 years I have taken part in the plant disease measurement work that your then President, Mr W. C. Moore, called for when he addressed you in 1941, and although we have gone far with that work since then, and I am sure it has been useful and solid enough (see *Some aspects of agricultural research*, No. 2, A.R.C., 1960), I have to confess, as I stand before you today, that I am still as much interested in mycologists as in mycology. Therefore I am going to revert, in this lecture near Christmas-time after a very busy year, to something more in the spirit of my *Advance of the fungi* (1940), and on this occasion I am going to speak not so much about plant pathology as about the higher fungi and our fungus forays.

We began 64 years ago as a company of field naturalists carrying on the forays of the old Woolhope Club. In 1919 our Plant Pathology Committee branched forth from its parent body, and now we are virtually two Societies: the new British Plant Pathological Society in all but name, and the old B.M.S. Our two parts mingle with complete compatibility and are fruitfully heterokaryotic. The fungi that it is our pleasure to collect, or by which we earn our living, have a curious fascination. They attract to our midst devotees from many walks of life: lawyers and businessmen, doctors and teachers, even excisemen and engineers. It was from this diverse company that I came in, and so I am going to speak today, not in the stilted language of professional mycology—but in any way I please, as an onlooker, an amateur, and an impertinent but very humble inquirer.

We are one kind of living organism observing another; so first I ask myself what is a fungus and then what are we? Gregory (1952) spoke of a fungus as the multinucleate protoplasmic inhabitant of a network of chitinous or cellulose tubes, which it secretes, and within which it moves. That was fine; rarely has so much been packed into so few words, and the term 'inhabitant' provokes our imagination. But it lights up only one aspect of our organism. The *total organismus*, as it extends in space and

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time (Fig. 1), has three parts. First a mycelium, growing hidden from our sight among the plant debris of the woods and fields, drawing some nourishment perhaps from the underlying soil, always competing with legions of other soil organisms, and sometimes living, as we know, in vital association with plant roots. This hidden mycelium, small or large as its individual colonies may be, is distributed over thousands of square miles in an environment beneath our feet. Here and there this lurking Gregorian inhabitant of the underworld thrusts up fruiting bodies, sporophores, toadstools—call them what you will—and these toadstools are the middle third of our fungus, and all that we commonly see. These visible toadstools are little gene-shuffling and spore-broadcasting stations, which produce and launch off the other third of the *total organismus*: an unseen cloud of spores, variably dispersed in the air.

Thus *Lepiota procera* is not something that we can pluck and put into a basket; it is all the mycelium of this species wherever it may be; it is all the successive crops of toadstools that this mycelium produces over the years; and it is all the drifts of spores that these toadstools release into the air. And in its successive generations it is never quite the same; there is a range of variation in its populations as it mutates by imperceptible degrees.

That is our fungus—the thing to be observed. Now what of us—the observers? We are creatures of flesh and blood looking out on an observable world; externally, and to each other, we may appear confident, pontifical, or even occasionally important; but within, in the privacy of our own thoughts and consciousness, each and every one of us is a poor, naked, lonely ghost. Of ourselves and by ourselves we are nothing. The limits of our being are our contacts with the minds of others, in the past, the present and the future. That is the first point I wish to make about us; and it explains why we gather together in this Society, how we provide incentives for the unpaid pursuits of Mycology, and why we so scrupulously maintain our immortalizing mystiques of publication and citation.

The other point I wish to make concerns the workings of our minds. We think always by analogy and are for ever inventing models. We erect these models to explain the unknown in terms of the known. We intend these models to be our servants, but all too often similes derived from them creep into our vocabulary as actualities, and they become our masters. Our minds, in so far as we are good modern scientists, are full of such models (Fig. 1): genes that have become the noumenons of idealistic philosophy: normal curves of error that masquerade as natural laws: creaky 'life-cycles' that leave out the whole of the variation upon which our revered model of evolution by natural selection is supposed to depend ...and so on. Now, in this year 1960, as we live in an age of electronic computers, we tend to think that our own very brains must be mere instruments for data processing, with ceaseless electric circuits through cellular memory stores. Our models and our surviving classicism produce a welter of high-sounding words, in which we clothe our thoughts or conceal our ignorance; but still, buried away in all this mass of mechanistic cerebration, are the scraps of poetry by which we live.

Gregory's 'inhabitant' of our fungus tubulations is pure poetry. It is a part of our credo, as scientists, that our fungi, like all other living things, are the inevitable consequence of a prolonged interplay of chemical and physical forces in the material fabric of the universe. They are the products of an automatism without beginning and without end. If we believe that, or we profess to believe it, or if we accept it, as we do, to serve the purposes of investigation, we should not speak of self-reproducing protoplasm as the

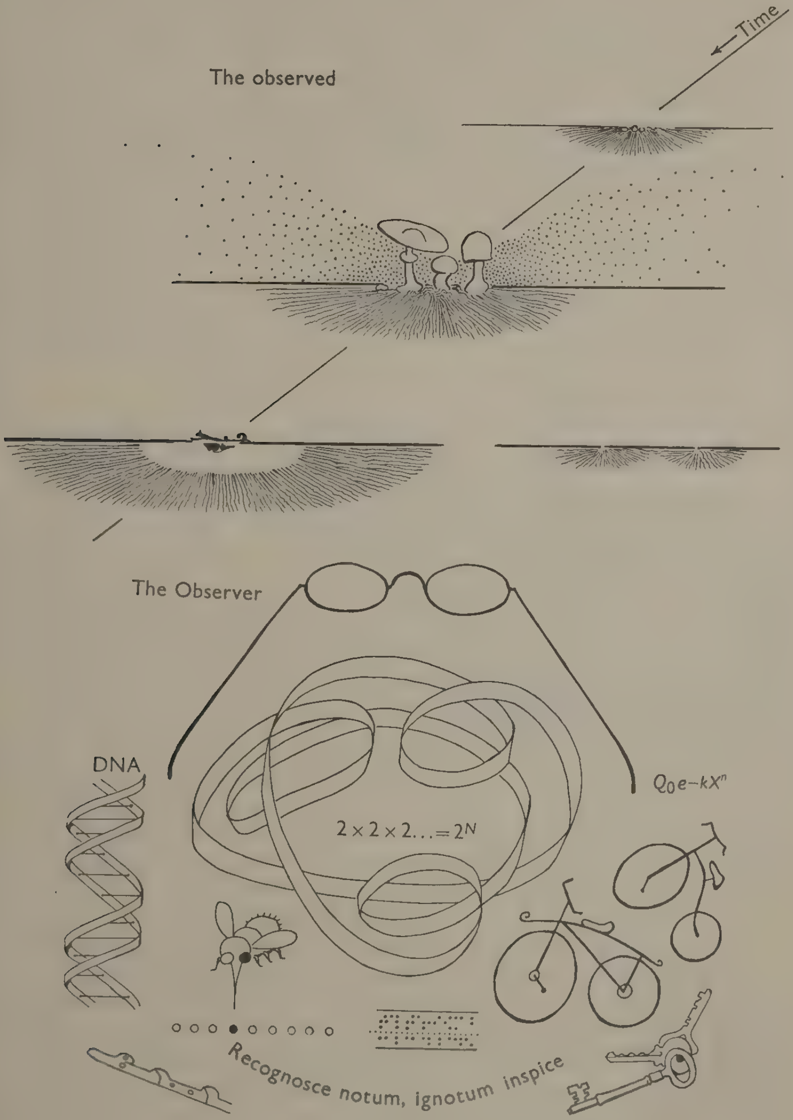

Time
The observed
The Observer
DNA
$Q_0 e{-kX^n}$
$2 \times 2 \times 2 \ldots = 2^N$
○ ○ ○ ● ○ ○ ○ ○ ○
Recognosce notum, ignotum inspice

Fig. 1. The Observed and the Observer.

'inhabitant' of our organism but as a 'constituent' of it. And indeed the word 'organism' is also wrong; we do not admit the postulation of any supernal power responsible for organizing fungus growth, nor do we credit the fungus with any volition whereby it could organize itself. The whole fungus—mycelium, toadstools and spores; below, on, or above the ground —is not an organism but a 'constituency'. That is the neutral term we need—if we can only cleanse it in our minds from its unfortunate association with politicians.

All through the past century, while we have been collecting and describing the middle thirds of our fungus constituencies, our fellow workers, the chemists and the physicists, have been probing into the nature of the vital constituents of living cells. Our fungus protoplasm, very rightly so-called, is a colloidal mass of proteins bathed in solutions of amino-acids, carbohydrates, inorganic salts and other dispersible contents of the cells. Here and there in the plasm are small sacs of equally gelatinous material which we call the cell nuclei. In these nuclei—and also outside them—there are nucleic acids; and it is these nucleic acids which are now believed to be the controllers of protein synthesis and thence the ultimate determinants of all living processes.

Among the many models in the workshops of our minds, there is now none more beautiful, or, as it seems to me, more likely to promote discovery, than the model of the DNA molecule constructed by Watson & Crick (1953). This model has stood its ground through 7 years of violent biochemical and genetical controversy (Mitchell, 1960; Pontecorvo, 1959), and it has the great merit of snapping together the findings of a very long train of workers—reaching back past Caspersson and Emil Fischer to the beginnings of organic chemistry—into a contrivance of such simplicity and inherent plausibility that we can all refer to it, and think by it, without much knowledge of the many and difficult techniques by which its parts were built up.

In this model two long chains of phosphorus atoms alternating with pentose sugar molecules, which have purine or pyrimidine bases attached, are rather loosely linked together in a double spiral by hydrogen bonds. There are hundreds of molecular units in each chain; but there are only two purine bases (adenine and guanine) and two pyrimidine bases (thymine and cytosine) in the whole structure. The purine groups are large, the pyrimidine groups are small. Each large group on one chain is linked with a small group on the other. Thus each half of the whole DNA molecule fits the other like a key in the wards of a lock.

The essential difference between one living species and another, and between one physiologic form of a species and another, is believed to be in the relative numbers and positions of the four bases on their respective DNA molecules. The molecules appear to carry the same base coding throughout all the cells of the organism of which they are part. They multiply—like chromosomes—by splitting in two; each half of the spiral chain then building on to itself its complementary opposite chain by picking up the necessary components from the solution in which it is immersed. At each division the order of the purine and pyrimidine groups is rigidly maintained—hence the fixity of our species; by occasional accident, or by X-ray bombardment, the order is disturbed—hence our mutations.

With this model in our minds life loses *one* of its mysteries. The self-copying of the vital nucleic acids is no more (or less) wonderful than the phenomena of crystallization. And the link between progeny and progenitor has been tracked down to the hydrogen bonds in the DNA molecule (Fig. 2). As we are mortal, and for us life is sweet, we speak affectionately of our fungi as living things, and the Greek word '*bios*' of which we are so fond is charged with a deep feeling that life is something beyond the ken of science, which passes from generation to generation of our fungi as it passed from Jehovah to Adam in the noble imagination of Michelangelo. Only occasionally do we draw back in time, to scratch out such words as 'inhabitant' and substitute 'complex biochemical processes'. Even then we do not go far enough. Science is a form of pragmatism, based on an 'as-if' philosophy. Now, for the sake of further discovery in a field that lies open to us, I think we should accept the showing of the DNA model that the study of our fungus constituents is not 'biochemistry'. It is just plain chemistry; if it is any special kind of chemistry it is colloid chemistry —there is no '*bios*' about it.

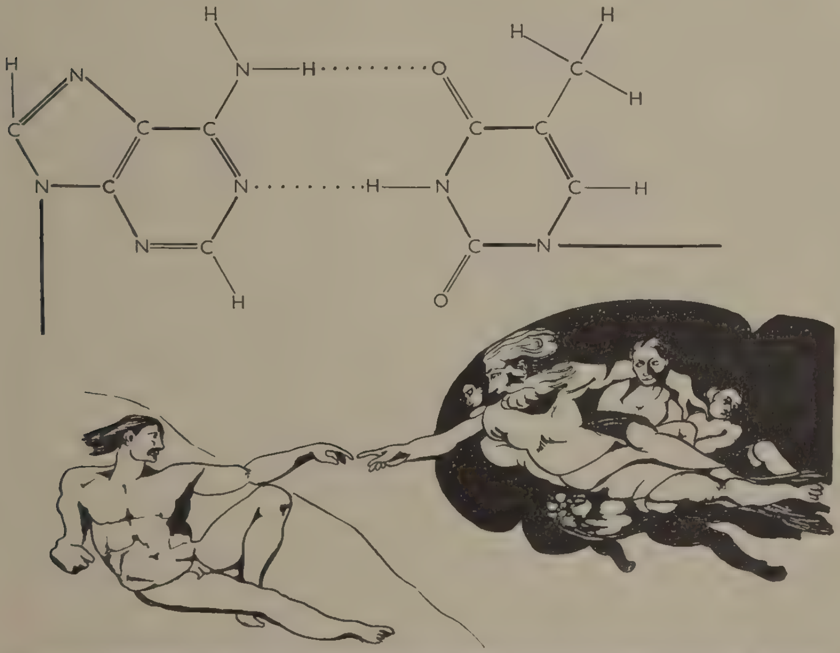


Fig. 2. Hydrogen bonds in the structure of the DNA molecule (after Watson & Crick, 1953); and the Creation of Adam (after Michelangelo, *ca.* 1510).

Science is an artificial way of looking at things, which has proved immensely successful in serving material ends. The indication we now have that every living thing is internally labelled chemically by the configuration of its DNA molecules is worth pursuing to the limit of our resources. The discovery has come from cancer research, where there is crucial need for more knowledge of molecular biology; a great part of modern academic plant pathology (Horsfall & Dimond, 1959–60) is now incomprehensible except in the light of nucleic acid constitution and protein synthesis. In taxonomy the concepts of 'molecular species' which are now emerging may soon make all the systems of classification we have had so far look like mere gropings in the dark. Back in the time of Liebig, acceptance of the naïve idea that plants needed feeding with the minerals to be found in them by chemical analysis, led to the establishment of our entire artificial fertilizer industry. Now that chemical analysis has progressed to a very much greater degree of finesse, the detailed chemical investigation of the nucleic acids and the proteins in all sorts of living things, animals and plants, fungi and bacteria, hosts and parasites, would be a great deal more likely to increase our material wealth than would sending sputniks to the moon.

Of that I am sure, but way down in my soul I am still not altogether convinced that either genes or DNA do contain all that steers a growing thing into the likeness of its ancestors. There is another point of view which we may find expressed, for example, by Eagle (1935) when he says 'all the germ-cell can possibly be is the material-to-start-on, with which some non-material entity begins the work of clothing itself with a physical duplicate of its own non-material nature'. Without going so far as to talk about non-material entities, I cannot help constructing for myself another model, in which those delicate electronic linkages in the DNA molecule act as valves responsive to ancestral influences of a kind that we have not detected. After all, there must be a very long train of directives of growth between the first division of the mycelium from a germinating spore and, for example, the expansion of a splendid Fly Agaric, with its red cap, intoxicating juices, and all its other attributes. I see no reason to assume that we have already discovered all the kinds of radiations, forces or influences operative among living things in nature. Why should we have done so? Historically our Science is very young.

There is also the small point that no one has yet actually determined the order of the nucleotide groups in the DNA of *any* living thing. It is fair to expect that this can be done; but it does not appear to have been done yet. And no one, it seems, has yet put forward any very convincing model of the way in which DNA molecules are built into the structure of chromosomes (Darlington, 1960). There is a very elegant model of the RNA in a tobacco mosaic virus particle (Holmes & Franklin, 1958), but some of the models of DNA in a chromosome (Steffensen in Mitchell, 1960; Taylor, 1957, cited in Anfinsen, 1960) look rather like early inventions (Fig. 3). A model which predicts that there are precisely twenty kinds of amino acid in cell proteins, and shows how these could be coded by groups of three nucleotides on the DNA chain (Crick, Griffith & Orgel, 1957, cited and discussed in Crick, 1958) is very elegant. But *we* know, by abundant experience, that as soon as anybody postulates that there are only twenty kinds of anything, someone else will promptly announce the finding of the twenty-first.

Perhaps it does not matter that there is no good model of the DNA in a gene. In *Trends in genetic analysis* (1959), we overhear Pontocorvo saying 'Finally, if there is any reality in the picture of the gene as an array of mutational sites, and if there is any reality in the picture of this array as a stretch of DNA duplex in which each site is a nucleotide pair, then there is open to experimental probe a very attractive possibility; namely, that the use of antagonists specific for each of the four kinds of nucleotides may produce specific mutations at each of the corresponding sites.' The old model of genes strung along chromosomes like beads on a string has to be discarded anyway. There is a hope that it may soon be possible to discard genes altogether, and to think directly in terms of real nucleotide groups on real DNA molecules. In one of the latest books on the cell nucleus (Mitchell, 1960), genes do not even appear in the index.

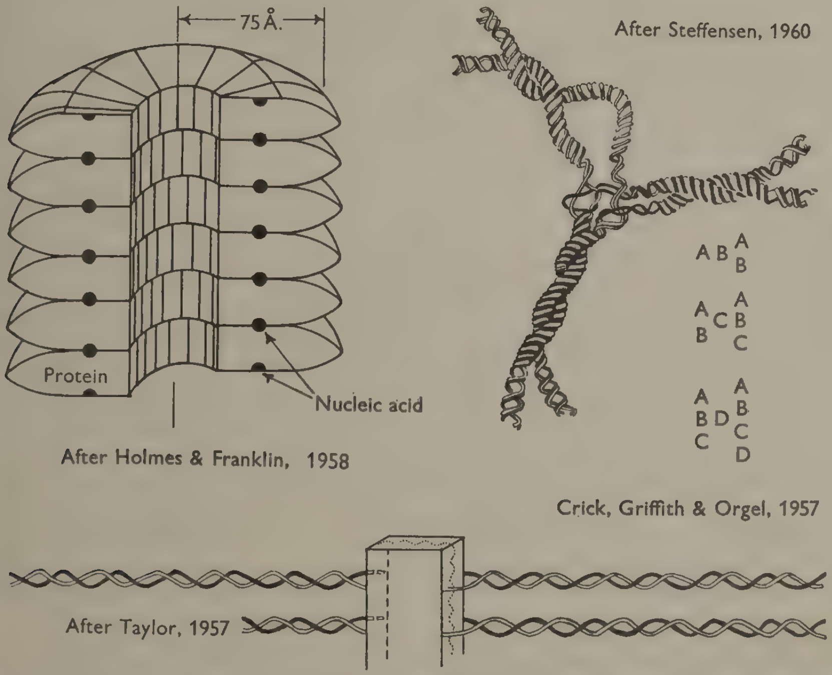


Fig. 3. Models of the RNA and protein in a tobacco mosaic virus particle, and of DNA chains in chromosomes, with combinations of four nucleotide groups (A, B, C and D) which could code 20 amino-acids in protein synthesis.

I have gone on rather long about the DNA model. That is because I believe it embodies the greatest clarifying conception in biology since Darwin. But when we stop to think of what must be happening in our fungus cells there is not only cell chemistry, there is also cell physics to be considered. I remember the quickening of interest I felt at one of our meetings when Dr Noel Robertson told us about his work on hyphal tips. He observed that when the tips of a Fusarium growing on nutrient agar were flooded for only 30 sec. with water their growth was momentarily checked, there was a slight swelling at their apices, and thereafter some of them put out branches below the swelling (Robertson, 1958) (Fig. 4, centre). In this work one small corner of a curtain was lifted. Can we not imagine much more? Take an internally increasing mass of protein jelly; let it form a skin, as it will, at its ageing boundaries. Then, with very little special constraint from its molecular structure, it will extrude in mycelial form; it will burst its bounds at points of weakness, and it will produce sometimes branches, and sometimes drops which have the very shapes of our fungus spores. The basal extrusion of spores can be well imitated, in one of my favourite Christmas toys, by letting drops of sodium alginate solution fall into lime-water. The apical extrusion of mycelium is precisely modelled by the outburst of branches from the gelatinous sac which envelopes a crystal of uranium nitrate dropped into water-glass solution (Fig. 4). There is much that the colloidal constituents of our fungi *must* do, willy nilly, as the inevitable consequence of the action upon them of osmotic pressure, surface tension, gravity, and other purely physical forces. We are fortunate in mycology, in having many good subjects for the study of protoplasmic architecture.

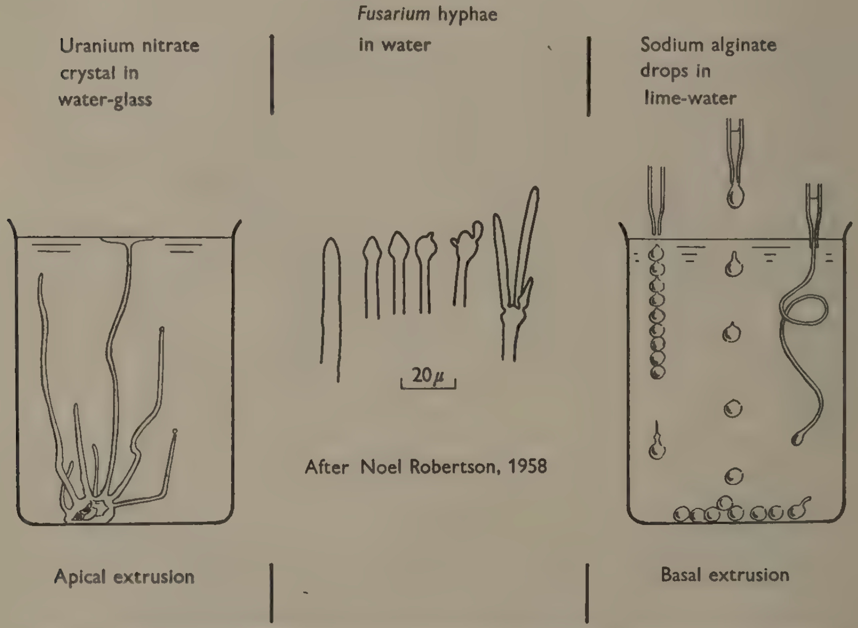

Uranium nitrate crystal in water-glass
Fusarium hyphae in water
Sodium alginate drops in lime-water
20µ
After Noel Robertson, 1958
Apical extrusion
Basal extrusion

Fig. 4. Chemical toys imitating mycelium and spore formation, shown in juxtaposition with a water-induced swelling and branching of hyphal tips.

And so we go on our forays, with plenty to think about, and plenty to discuss, when the rain stops the collecting and we sit eating our lunch under some autumn-tinted tree. Sometimes on such occasions we *do* talk about mycology, and that very learnedly, but the discussion is usually about the naming of our finds. For the rest we pluck our pretty toadstools and put them in our baskets, without a thought to the way they grow, to their acres of spawn, or to their spreading clouds of spores. In a sense this collecting and naming of fungi according to the characters of their sporophores is about as scientific, and about as innocent, as the enthusiasm of our Victorian great-grandfathers for collecting and classifying sea shells without regard to the marine animals that were once *their* inhabitants. But there is more to our forays than mere collecting, and more to the *logos* of mycology than those parts which we regard as scientific.

My wife is even less of a systematist than I am, but she loves the fungi. It is her pleasure to line small baskets with moss, and then deck them with sundry small puff-balls and colourful agarics to give to our neighbours when they are ill or troubled. She is also a teacher, and often, as the autumns have come and gone, she has captivated both her troops of small children and their parents, by getting them to bring in all the different sorts of fungi they could find in the fields and spinneys about their homes. Teachers know that an interest started in childhood may enrich a whole life. And then, if you will forgive me, I will take my own case. I like the fungi for the wealth of anecdote and legend with which they are surrounded (for a treasury of this, see Ramsbottom, 1953). I also like them because I am fond of irony. I like to contrast the vainglories of Blenheim Palace with the charm and beauty of the *Boletus satanus* which is to be seen, free of charge, growing under the old oak trees by the lake. I like our toadstools for their transience, for the very reason that (save by the remarkably successful freeze-drying of Davies, 1961) they cannot be kept or accumulated; because they rot; because their images have to be preserved in mind and memory. I like the part of them that utterly escapes imprisonment in the dreary morgues of pickled vegetables, smelling of floor polish and clove oil, in our botany departments and museums. I like them, in short, as objects of grace. Others, I know, like them for other reasons. Some even like them to eat, and perhaps our mycophagists are the best mycologists of us all, for they know their fungi by all-in biologic assay. For them, picking esculents among the Amanitas, as in Madame Le Gal's *Promenades mycologiques* (1957), it is often a case of your mycology or your life.

But in asserting that our interests in the fungi are as diverse as the people who take part in our forays, I must not stray too far. The recognition, recording and naming of the fungi has been, historically, our first charge, and without our systematists the whole Society would collapse. We have no gold mace to lay out at our meetings, but we do have a famous old Foray Box that goes before us to the workrooms of our spring and autumn forays. In this box are the few precious books and other publications that are really useful for running down our fungi. Last year's addition to the box was Moore's *British parasitic fungi* (1959); this year's has been the magnificent 'New Check List of British agarics and boleti', by Dennis, Orton & Hora (1960).

Sometimes, while others are busy examining cystidia, I sit gazing idly at our foray box. It conjures up for me a vision of a great book, a ghostly *Flora fungorum*, wherein are inscribed all the known facts about the fungi,

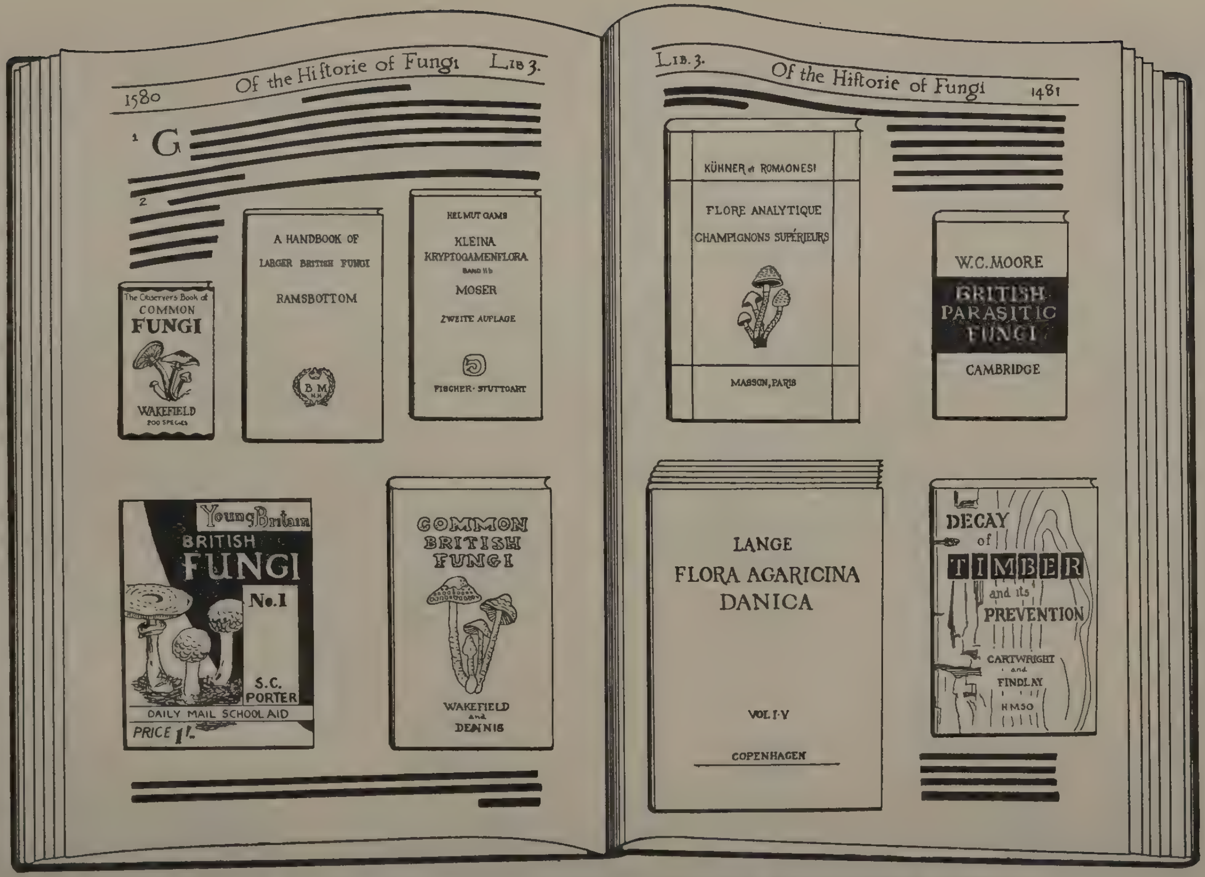


Fig. 5. Some important books from the Society's Foray Box shown against the outline of an imaginary *Flora fungorum* in the likeness of Gerard's Herbal.

as a part of Man's inventory of Nature. I sit in awe of the religious impulsion that makes men devote their lives to this recording—although most of it is accessible only to a small priesthood working in the great museums. In the foray box, besides Lange (1935–40) which now costs about £60, we have incidentally, the minimal equipment of books for a modern amateur agaricologist (Fig. 5). This includes Ramsbottom (1923), revised 1944, costing only 7*s*. 6*d*., which calls things by their old names, but is still the best arranged, and invaluable for the ascomycetous oddments at the end; Wakefield & Dennis (1950), costing 35*s*., helpful but bulky and covering only 470 species; Kühner & Romagnesi (1953), a great work but in French, with keys based on microscopic characters, and decidedly expensive (about £8); and Moser (1955), portable, crisp, covering most of the species and that by their latest names, but in German, costing about 28*s*.

On the basis of his statistics, Parker-Rhodes (1955*a*) has said that even after 100 years of our foraying we probably have not yet found anything like 99% of the fungus species that there are in Britain. The 1696 species or varieties named in bold type in the New Check List are perhaps enough to go on with. The great work of cataloguing our agarics and boleti can never be complete but it is very far advanced.

Now we want a flora, in English, covering everything named in the New Check List. We want a book that will bear the same relation to the whole literature on these fungi that Clapham, Tutin & Warburg (1952) bears to the whole literature on the flowering plants. The New Check List is the tantalizing prospectus of a yet unwritten book.

To my mind there is a very clear distinction between the devout work of making a true inventory of our fungi, and the pleasures of sorting the entries in this way and that, according to ever-changing conceptions of taxonomic differences. I tend to think of this shuffling of the cards as being in part heuristic—in so far as it stimulates the comparative study of fungus anatomy and constitution; but in part, and perhaps chiefly, the endlessly enjoyable pastime of a particular kind of scholar: a kind of demon-patience demanding acute powers of observation and an enviable love of languages, mostly foreign or dead.

And here I come to the matter of naming our fungi. The names in the New Check List are fine for those few of us who wish to delve into the history of the transference of a species from one genus to another, and the doubtless very good reasons for its present position. They are not so good for those of us who merely want to name our finds, and thereby go home with the pleasant illusion that we know them—and have somehow got them in the bag. We do not mind a bit of Latin and Greek. Some of the old familiar binomials—*Mycena pura* and *Marasmius oreades*, for example—are quite euphonious, and we like to roll them round our mouths like fine old brandy. It distresses us to find our *Armillaria mucida* changed into something we have not yet learned to pronounce. But we can get over this. There is only one *mucida* in the New Check List, so the specific epithet alone will serve as the whole, complete and distinctive name for that particular watery toadstool that grows on the branches of trees. We can go on strike, and ignore generic names altogether, leaving them to be the playthings of the wrangling systematists—provided they leave the specific epithets alone.

It is in good *common* names for our fungi that we are most poverty stricken. It is all wrong that almost every one of the 520 listed 'British' birds should have a good English name, while so many even of our commonest fungi are nameless in our mother tongue. Miss Wakefield (1954) with her five-shilling '*Observer's Book of Common Fungi*' and Mr S. C. Porter, with his two admirable shilling pamphlets on *British fungi* in the Young Britain Series (1946–47), have shown us that when our fungi can be given sensible English names, and they are illustrated by good photographs and described in plain language, they can be introduced to children in school. If we have any regard at all for human values, such sound and inexpensive publications for children are just as important as monographs for museums. If we want to lay bait for more Pearsons and de Barys, and we want to reach the future guardians of our diminishing nature reserves, then we must look to the children. The halcyon days of childhood are short now, with the grey grind of competition for privilege beginning before eleven. We want names for the fungi that young lips can pronounce. Not long names with awkward Latin plurals, like 'Lactarii' or 'Lactariuses', but good short rude names, like 'puff-ball' and 'stinkhorn'.

I have noticed that this Society has a way of producing unpretentious 'Lists' which prove on examination to be pieces of creative writing. The *List of common British plant diseases* (1944) was much more than a mere list—it helped plant pathologists to put their whole house in order. Could we not next produce a List of Recommended Common Names for the Higher Fungi?

Common names may last for centuries if they are good enough to win their way into the vernacular. Scientific names, as we have seen, change with every fashion in taxonomy. Just now classification by microscopic characters is all the rage, although many of these characters are not a whit more fundamental than habitat, and macroscopic form, and taste and smell. By about the year 2060, one of our future Presidents may be saluting yet another Check List, from which all our genera and species have disappeared leaving our fungi classified solely according to the chemical constitution of their cell contents. The problems of nomenclature will be no less vexatious then than they are now; but a stinkhorn will still be a stinkhorn, and a puff-ball a puff-ball.

So far, in all that concerns our fungus floras, and the naming of our fungi, I have been speaking, as we usually do, as if there were nothing to a fungus but its sporophores—as if toadstools were all. Now I am going to widen the field, and get away from the mere middle-third of our fungus constituencies, by asking an innocent question. What is there of any special interest about a rare fungus?

If it is rare it is not playing any significant part in the balance of Nature. It may be important as evidence that the nucleotide groups in fungus material can combine in yet another way. It may help to fill in somebody's floristic pattern or evolutionary dream picture. But do these things really matter very much? The question I think we should ask is: *why* is this fungus rare?

If it is not a recent sport or mutation, why hasn't it spread? Why is it inefficient? Has it a defective mechanism of dispersal? Or has it some other handicap that prevents it from colonizing new territory?

I have tried this question on several of my learnéd friends during this year, and they have set about me like wolves. They have not liked my suggestion that anything in Nature could be inefficient. That, it seems, is blasphemy.

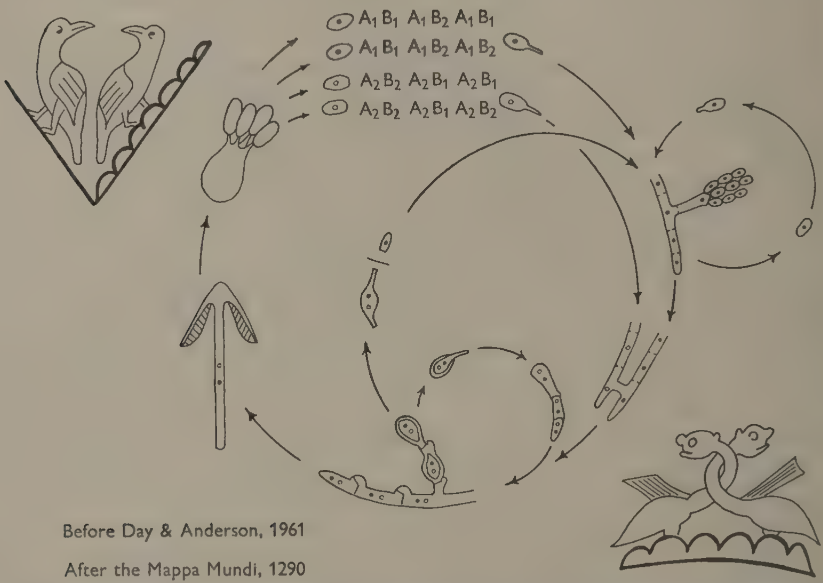
Dr Lilian Hawker has reminded me that many fungi can, and probably do, grow quite extensively and quite efficiently as sterile mycelium; and it may be only when adverse conditions disturb this blissful efficiency that they are provoked into producing sporophores as a means of survival. I am grateful for that facet of truth; it leaves me all the more interested in the mycelium of our fungi; but it hasn't answered my question. Then I have had a free lesson on the Gregorys' spore dispersal equations (Gregory, 1945), from the authors thereof, and they have convinced me that although the vast majority of the spores from an agaric come to the ground in its immediate vicinity, some do undoubtedly get away and become airborne for quite long distances. I am left wondering what feeds on all those spores which appear to be 'wasted'; and I see that the odds against any individual spore reaching a distant ecological niche are very great indeed. But even if the odds are a billion to one I also see that any efficient fungus will get over this by producing a billion spores or more. It will permute everything by everything in the lottery for life. And lastly, Dr Peter Day at John Innes has very patiently tried to explain to me the intricacies of his work on the genetics of *Coprinus lagopus* (Day, 1960). He has confronted me with the fact that many, and perhaps the majority, of our agarics are heterothallic; which I have taken to mean that they need mycelium from not one, but from two genetically different spores before they can establish a fruiting colony (Fig. 6).

The possible consequences of this last have intrigued me. Suppose, I say, that there is still a small uninhabited island somewhere in the remote Pacific. Sooner or later some shipwrecked mariner will be washed up alive on this island, and then Man will be present on it. The odds against such an accident are very great, but that is not all; before a human family can possibly be running about on the island another very rare accident has to occur. Shall we say there has to be a plane crash that deposits an air-hostess in the area. The odds against the shipwreck and the air crash occurring in the same place and in the same lifetime are so fantastically great that the island stays unpopulated until the end of the world.

Is this why *Strobilomyces strobilaceus* (*S. floccopus*, New Check List) stays confined to its same few sites in our woods each year? Is it because it is heterothallic (if it is) and the odds against *two* of its genetically different spores alighting and germinating on just the right scrap of nourishment for them are not a billion, but a billion times a billion to one? And that such odds are therefore too great even for the prodigality of Nature?

I do not know. I do not know enough about genetics to say whether it is possible for the mycelium from a single spore of a heterothallic fungus to mutate and produce its other half (as Eve was made from a rib of Adam). What I can see is that it is not in our knowledge of the taxonomy of our toadstools, but in our knowledge of their populations, of the fate of their spores, and of the whole life history of their mycelium underground, that the great yawning gaps appear. My simple question, 'Why is this fungus rare?', soon brings us to the chill and misty frontiers of the unknown; and to the awful injunction in the motto of our Society: *inspice ignotum.*

But our explorers are out now, and out in many directions. The air party, led by our gentle and far-seeing philosopher, P. H. Gregory, and his complementary man-of-action, Dr Hirst, with those fine workers on the medical side, Dr Hyde and Mrs Adams at Cardiff, have gone beyond Ingold's researches on spore launching (Ingold, 1953) and indeed broken into the turbulent air. Gregory's forthcoming book, *The microbiology of the atmosphere* (1961), will tell how the pursuit of air-borne spores and pollen was taken up again, from where it was left by Pasteur, and by Miquel in Paris (Miquel, 1883). It will lay open a new chapter in the history of medicine and plant pathology. It will tell of the Manchester physician, Blackley (1873), who sent traps up into the air on kites and caught the pollen grains that he associated with hay fever, and who also gave himself acute bronchial catarrh by inhaling fungus spores. I can tell you this book is one that you will read twice, first, for its human interest, and later, for its facts and figures, as a compendium of working information about aerobiology.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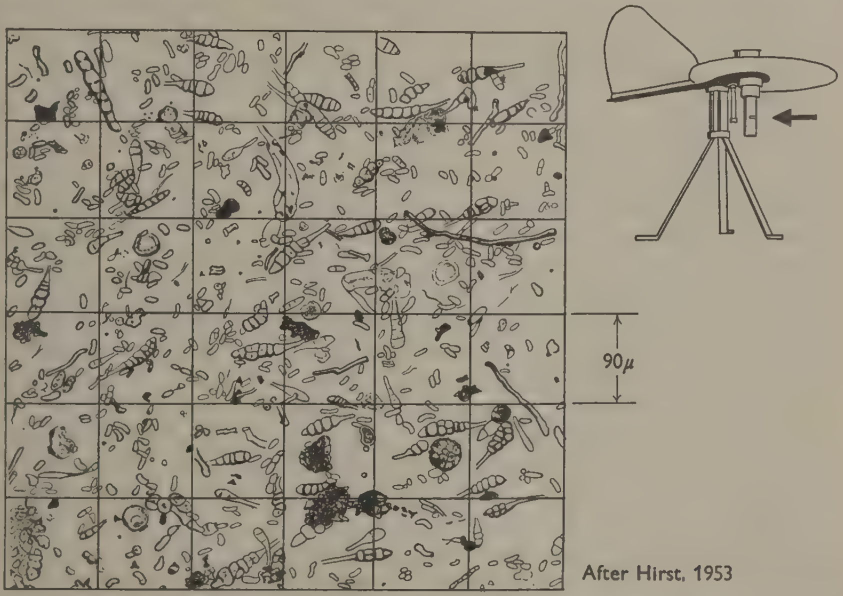
A_2B_2 A_2B_1 A_2B_2

Before Day & Anderson, 1961

After the Mappa Mundi, 1290

Fig. 6. Life cycle of *Coprinus lagopus*. If two monokaryons have different alleles at each of two loci, A and B, they are compatible and can exchange nuclei by hyphal fusion to form dikaryons. The devices symbolic of coupling and segregation in the life process are from the Mappa Mundi in Hereford Cathedral.

All I am going to do now is to comment on the new kind of fungus foray that is being made with the Hirst spore trap (Fig. 7). Hirst (1952) cut the cascades out of the May cascade impactor; he put the trap under suction; turned it into the wind; and made the sticky trapping surface travel automatically past the orifice, so that the changing air spore-load of a whole day is sampled hour by hour on a single microscope slide. With this new instrument of exploration, spore forays can be made in different localities, at different altitudes, and at different times of the day and of the year, and the catch is always from a known volume of air (Hirst, 1953). Slides from this trap have shown beyond question that the spores of our basidiomycetes are widely dispersed in the air, and, incidentally, they have shown a totally unsuspected abundance in the air at night of minute hyaline basidiospores of a species of *Sporobolomyces*, a 'mirror yeast', which grows on the surfaces of leaves (Last, 1955).



After Hirst, 1953

Fig. 7. The Hirst spore trap, and a small part of the spore catch near a wheatfield on a warm windy afternoon in early August.

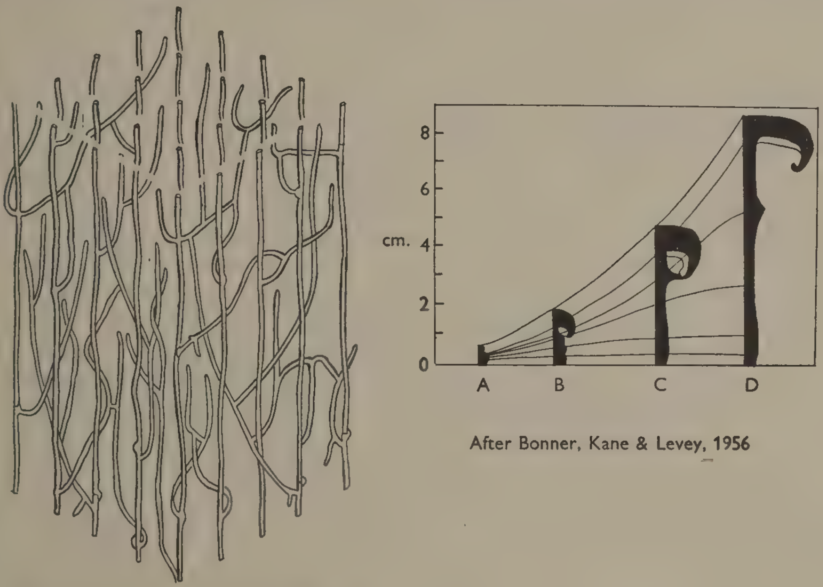
With the Hirst trap the spore collections of a few days are enough to provide spore-foray material for an entire winter. And unlike our forays of toadstools, these spore forays are both qualitative and quantitative. When the slide-scanners find the spores of *Ganoderma applanatum*, they do not just leave it at that; they record the precise count of such spores per cubic metre of air.

Hyde & Adams (1958) gave us an atlas of air-borne pollen; Gregory (1961) is giving us the beginnings of a corresponding atlas of fungus spores. Already the investigation of what he has called the air 'spora' has yielded information of great importance; in human pathology, about asthma and other allergy diseases (for review see Hyde, 1960); in plant pathology, about apple scab (Hirst, Storey, Ward & Wilcox, 1955), and, as we heard with spell-bound attention at our last paper-reading meeting, about the black rust of wheat (Ogilvie, Thorpe, Hyde & Adams, Hirst and Hogg in *Proceedings* of 1960, these *Transactions*, **44**, 136, 1961). The equations put forward by Gregory (1945) for the expected spore concentration in the air, and the expected rate of spore deposition from it, at varying distances for the source, are being tested, and so far have proved almost prophetically correct. Aerial spore transport from country to country is now being related to the trajectories of the winds.

If anyone would like a telling story about the upper third of one of our foray fungi, it is to be found in the Forestry Commission's most recent account of *Fomes annosus* in Great Britain (Low & Gladman, 1960) and in the beautiful investigations of the dispersal of this fungus that have been made by Rishbeth (1959). One of Dr Rishbeth's methods has been to expose freshly cut sections of pine stem at various distances from plantations in which the sporophores of this *Fomes* are sporing on old stumps. The fall-out of spores from the air load reaches the exposed wood, miles from its source, although the stem sections are only a few inches in diameter, and where the spores alight they germinate and grow, producing colonies of the fungus that can be counted. This provides a model of the way in which unprotected stumps become infected in forestry practice. It is from these stumps that the fungus spreads slowly through old roots underground to reach the roots of standing trees. The investigations have shown that the immediate protection of cut stumps when the plantations are thinned is an effective and inexpensive measure of control.

The creeping of the mycelium of *Fomes annosus* from tree root to tree root underground brings me to the lower third of our fungus constituencies. It is of this part of their existence that we know least. Occasionally, as in the study of root invasion, or in the production of rhizomorphs by *Armillaria mellea*, there are parts of this subterranean life that can be followed fairly easily; but the mycelium of most of our toadstools evades observation. We come across a troop of toadstools in some place, and we gather maybe a pound or two. We know, if we pause to think about it, that somewhere thereabouts, in the dead leaves or in the soil, there must be, or there must have been, an expanse of mycelium commensurate in bulk with the sporophores it has produced. And yet, if we rake among the leaves we find almost nothing of it—a few glistening scraps and threads if we are lucky, rarely more. Exploration by trapping and plating-out techniques has shown that the soil is alive with fungi. In *An introduction to the biology of micro-organisms*, Lilian Hawker, Linton, Folkes & Carlile (1960) say it is estimated that there are about 400 m. of fungus mycelium per gramme of a fertile soil (that comes to about 7 *miles* of mycelium per *ounce*), but most of this is contributed by sundry moulds, predominantly by species of *Mucor*, *Penicillium*, *Trichoderma* and *Aspergillus* (Chesters, 1949). Warcup (1959) and others have succeeded in rearing a few basidiomycetes from mycelium in soil fragments. Parker-Rhodes (1955*b*), and others to whom he refers, have given much thought to the kinetics of fairy rings, and drawn inferences about the hidden mycelium from the way in which these rings of visible toadstools slowly widen year by year, leaving barren zones behind. But no one, so far as I know, has yet been able to map the actual growth of the mycelium of any toadstool in the soil.

It seems to be generally agreed that the sporophores start as tiny knobs or buttons here and there on the mycelium; and Bonner, Kane & Levey (1956) do provide a model, from the common mushroom, of the way in which the sporophore enlarges from this button stage (Fig. 8, right). It appears that all the cells in the button are laid down when it is very small, and that the structure then expands by the growth or filling out of these cells with material supplied by the mycelium. Buller (1931) provides a model of an earlier stage in the process, at least for *Coprinus sterquilinus* growing on dung balls. He shows the mycelial filaments, sometimes from many spores, elaborately interconnected in a three-dimensional network (Fig. 8, left), so that when a fruit-body starts to form on any part of the mycelium, the whole of the rest can participate in supplying it with material. But what passes from the network into the button? It is not just water, for Bonner and his co-workers say the wet-to-dry-weight ratio remains constant in the developing mushroom. Is it protoplasm, then, that goes in? Or is it a diffusible, and only partially elaborated, nutritive solution? Where is the material stored to give such a rapid supply? And how is it moved? As up a wick, or within the hyphae? By mycelial pressure or by evaporation suction, as Plunkett (1958) seems to suggest? Does the stuff creep like an amoeba—or flow like sap? Are there mains by which it reaches the button? And are they ring mains or grid mains? How



After Bonner, Kane & Levey, 1956

Isometric projection based on Buller, 1931

Fig. 8. Three-dimensional network of mycelial filaments in *Coprinus sterquilinus*, and (right) stages in the development of a common mushroom from the button stage.

long does the process take? At what time in the season are those small knobs formed, which fill out so prodigiously to give us our troops of visible toadstools in the autumn?

It seems that even truffle hunting in England is beset with less inherent difficulty than the study of the mycelium of our commonest toadstools. Since the Belfast foray in 1948, Dr Lilian Hawker with her students and friends has made over 700 separate collections of British hypogeous fungi, and she has produced a finely illustrated monograph on them (Hawker, 1954) which I have quietly put on my shelf beside the *Carpologia* of the brothers Tulasne; but the characteristics, the forms, the organization, and the whole life-history of the hypogeous parts of our toadstools remain, for the most part, still unfound, unfigured and unsung.

It seems to me that there is only one really important kind of information about the lower thirds of our foray fungi that has so far found its way into our fungus floras, and that concerns the kind of material on which the mycelia grow. Often we do not know whether a particular mycelium grows on leaf debris or on roots, but at least the association of our woodland fungi with particular trees has been well recorded. That is something, and chemically speaking, once we get down to the analysis of the constituents of our fungi, and of their nutrient requirements, it may prove to be a very great deal. Our mycological forefathers may have been wiser than they knew in insisting upon association as a diagnostic character in our agarics.

Parker-Rhodes (1955*b*) speaks of a fundamental property in the physiology of our fungi which makes impossible the continued growth of mycelium in the same body of medium. Dr Dobbs, with Dr Hinson and his other co-workers at Bangor (Dobbs & Bywater, 1959), have found a very significant inhibition of fungal growth in soils. Apart even from the ruthless competition of other soil organisms, life cannot be easy for the mycelia of our fungi. There is one thought, which I have found in Buller (1931), that seems to bear hard on my question: why is this fungus rare? He speaks of the 'social organization' of fungus mycelium: of the co-operation of mycelium from many spores in effecting the production of *Coprinus* toadstools on his dung-balls. Dr Garrett, in the field of plant pathology that he has made his own, finds that a certain 'inoculum potential' (Garrett, 1956) is necessary before his soil-borne plant pathogens can bring about infection. Here Garrett and Buller seem to have said the same thing in different ways.

When it comes to colonizing my desert island in the Pacific, there is need for more than my air-hostess effect. We all know that if we really want to populate a desert island, one shipwrecked mariner and one air-hostess are not enough. In practice it is necessary to send out a whole shipload of people, with plenty of foodstuff to give them a start, and all sorts of miscellaneous gear.

But let me return to our forays. When we go out collecting fungi, we cannot very well take with us any apparatus for paper chromatography; we cannot do much with spore-traps (although one of Gregory's (1954) is portable enough); we can hardly stop to plate out soil fragments for the study of mycelium. There must be a division of labour. We must look to our research workers for enlightenment about those parts of our fungus constituencies which are hidden from the naked eye; and they, I think, should rightly look to us for the kind of information that can properly be obtained by field naturalists in the field.

And so I ask whether there is any more that we can get from our sociable forays and from our individual collecting than we have got in the past. I do not like to talk about physiology, ecology and phenology. These are so often mere pie-crust words rolled out to cover very little meat. What I want to say is that, by systematizing and pooling our observations a little better, I am sure we could obtain more information about the populations of our fungi. Not only about their distribution, but about their relative abundance or scarcity in different terrains and at different times of the year; and on the relation of toadstool production to seasonal weather conditions.

We have had a classical series of papers on a number of the statistical aspects of this kind of study from Parker-Rhodes (1953, 1957), who made pioneering and very detailed observations over a number of years on Skokholm Island and in Madingley Wood. The fine piece of direct experimental work on toadstool production in woods at Mortimer, near Reading, that Dr Hora made the subject of his Presidential Address in 1958 (Hora, 1959), is still fresh in our memory. Would that I had anything by half as substantial or as interesting to give you about our foray fungi! But still I say that as a Society we have sadly neglected to record anything for the future about the numbers of our agarics. In some years we have said it was too cold for them, in others that it was too wet or too dry, but nowhere in our records is there the slightest indication of how many or how few representatives of any species we saw in any year. Our foray lists bear no markings at all for frequency or abundance. To me, looking upon our New Check List, it seems an astonishing thing that we have thought it important to record all the synonyms of *Pholiota abstrusa* back to 1838, but not to record any tally of this, or of any other species, in 1958, or indeed in any year at all.

Tansley (1946) gives frequency symbols widely used in British lists of flowering plants and suggests that, in an afternoon's foray of a piece of woodland about a mile square, *a* (abundant) would mean never out of sight; *f* (frequent), constantly being met with; *o* (occasional), seen ten or twenty times; and *r* (rare), seen once or twice. Records in such general terms as these would be better than nothing; but I am sure that with a little pacing out and mental arithmetic, and a little mycological common sense in matters of definition, it would be possible to devise an approximately logarithmic scale, and so to record whether the number of toadstools of any species, in any foray area, and on any occasion, was apparently *nil*, or of the order of 1, 10, 100, 1000 or 10,000 or more per acre.

Once such assessments of toadstool populations had been made, on a sufficient number of occasions, and in a sufficient number of places, they would provide measures, or parameters, of toadstool production which could be correlated with already available parameters of climate and weather (Fig. 9). Perhaps only those of us who have engaged in plant-disease assessment work can realize to the full how great are the resources of our friends in the Meteorological Office for providing the kind of meteorological information needed in mycological investigations. They can provide not only raw data on temperature and rainfall, but also much more elaborate and searching parameters, such as potential transpirations and soil moisture deficits (Smith, 1958; M.A.F.F., 1960).

Perhaps the measurement of toadstool populations may appear very difficult. But then the measurement and quantitative surveying of plant diseases did not appear particularly easy, when it was started by Buddin and Moore and the other stalwarts on this Society's Disease Measurement Committee, back in 1941. Little by little in the years since then some of the difficulties have been overcome, and the work in this field, which has been pursued since 1950 by the Plant Pathology Laboratory at Harpenden and the National Agricultural Advisory Service, has provided full or partial quantitative information about quite a number of plant diseases in England and Wales (A.R.C., 1960). One paper which I compiled, and which I like to remember (Large, 1958), shows how disease measurements were used to obtain estimates for the whole country of the losses caused by potato blight over a 10-year period; another (Large, 1961) extends this, and gives similar estimates for common scab of potatoes, caused by *Streptomyces scabies*.

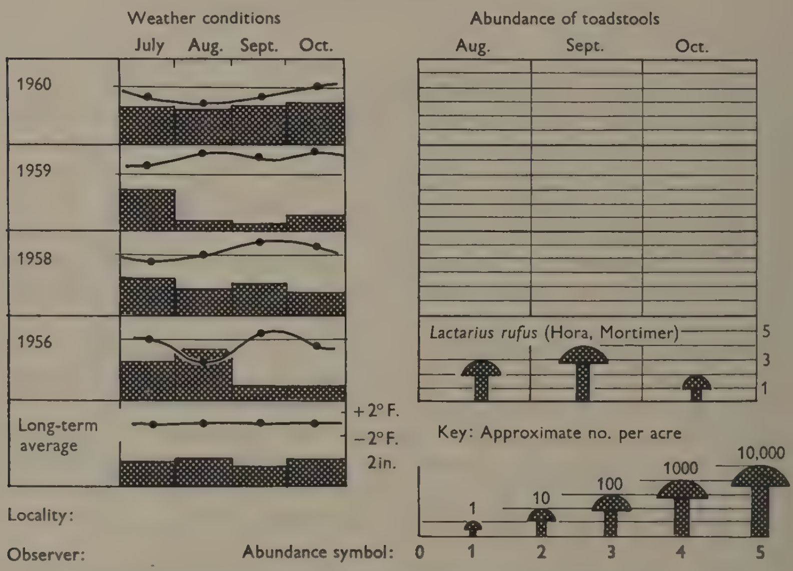


Fig. 9. A form for recording the abundance of toadstools in relation to seasonal conditions, with the lines of a suggested key; and specimen entries taken from Hora (1959).

In our work on the plant pathological side, when we have to tackle the measurement of yet another plant disease, we follow certain now well-trodden ways. First, we spend a good deal of time getting out a practical field key for the disease that anybody can use. Then we look around for ways and means of getting the key used in crop surveys that are already being made for other purposes. We also look for ways of relating the findings from widespread surveys to the results of a limited number of carefully conducted field trials (Large, 1955).

Can the ruses used in disease measurement be adapted or applied for exploring the populations of our toadstools? I can hear the objection that there is not the economic incentive. Plant diseases spell money: money lost, money made, money earned and money saved. Toadstools (excepting cultivated mushrooms) do not spell money; they belong to the unprofitable fairylands of natural science, literature and art. But that worldly wise and humanly despicable distinction is not entirely true. Quite a number of the higher fungi, even if none of them are of any immediate use for the national drug parlour, certainly do matter in commercial forestry, And here, as on our forays, our plant pathologists and agaricologists meet under the trees.

Armillaria mellea, which Dr Hora calls 'the mother of confusion', is not only a very common and most interesting toadstool; it is also a plant pathogen that does a great deal of damage. The polypores, that so often give us a good foray when the gill-fungi are scarce, are important agents of decay in cut and standing timber. Unfortunately we still lack a good field book for the identification of the polypores; and this is a pity—for they are among the most interesting of all the higher fungi. There is a satisfying completeness about our knowledge of them, in that their lower thirds have not been ignored. It is their mycelium that causes the decay in timber, and in this medium their mycelia have been studied and described (Cartwright & Findlay, 1958). At the Forest Products Research Station many of them are grown successfully in culture, even to the stage of producing their fruiting bodies. And then in the woods there are our charming boleti, many of them known to live in mycorrhizal association with the trees; and some of them no doubt affecting the establishment of new plantations, or the rate of growth of timber in old ones.

When I read in the Forestry Commission's Code of sample plot procedure (Hummel, Locke, Jeffers & Christie, 1959) that the Commission have more than 700 permanent sample plots, of 0·2–0·3 acres, in plantations distributed over all the afforestation areas of Great Britain; mostly in conifers but also about 100 in broad-leafed species; and that on these plots everything concerning the growth and management of the trees is systematically recorded over the years; and that on these plots the density of ground vegetation is also recorded, and recorded with frequency symbols—then, I ask myself: why not also the fungi? Why the mosses and the liverworts but not the fungi? Are the mosses and the liverworts, as a group, very much easier to identify than the fungi? I doubt it. And I say to myself: how wonderful it would be if only a short list of easily recognized agarics, and boleti, and polypores, and pezizas, could be recorded, for presence and abundance, on all the dates of inspection, on all those 700 sample plots about the country, every year. It would be something like a survey!

If it is said that foresters are brawny men, more inclined to be hewers of wood and haulers of timber than field mycologists; then I say there is all the more point to my plea for awakening more interest in the fungi in our schools. Foresters also come from schools. And if there is difficulty in finding good ways of assessing the abundance of sporophores; then that also is a challenge to us, for it is a necessary part of mycology if we are ever going to study the distribution and the life-histories of our species, as well as the characters of their sporophores and their mere names.

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Trans. Brit. mycol. Soc. **44** (1), 24–26 (1961).

LEPTOSPHAERIA ELAEIDIS SP.NOV. ISOLATED FROM ANTHRACNOSED TISSUE OF OIL PALM SEEDLINGS

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West African Institute for Oil Palm Research, near Benin City, Nigeria

(With 1 Text-figure)

Isolations from anthracnosed tissue of oil-palm seedlings developed acervuli of a *Pestalotiopsis* followed by perithecia of a *Leptosphaeria*. Single ascospore isolations from the latter showed these fructifications to be different states of the same fungus.

In Nigeria oil-palm seedlings frequently suffer from a severe necrosis when they are transplanted from raised trays (prenursery) to the open nursery. Their age is then approximately 5 months. This necrosis is thought to be due to transplanting shock, though it is more severe when overcrowding has occurred in the raised trays.

When a series of isolations from the anthracnosed lesions of these transplants was made, a number from one lesion produced a *Pestalotiopsis* species. This particular lesion was typical of that caused by *Glomerella cingulata* and measured 3·2 × 1·5 cm. The central area, which had already dried out and become papery in texture, was surrounded by a dark brown zone which in turn was surrounded by a diffuse yellow halo that gradually merged into the green colour of the leaf.

Isolations were performed by the following procedure. The whole lesion was surfaced sterilized with mercuric chloride, washed in sterile, distilled water, and cut into strips about 0·5 cm. wide. These strips were then placed on separate plates of 2 % potato dextrose agar. As expected, a *Glomerella* developed in most of the plates, but in four, acervuli of a *Pestalotiopsis* were formed. Pure cultures were obtained as re-isolations from these acervuli and it was in these cultures that the perithecia of the *Leptosphaeria* were first found.

A further examination of the plant tissue showed that acervuli of the *Pestalotiopsis* had developed in the centre of the papery tissue. Later attempts to inoculate this fungus into healthy leaf tissue all failed, but it was successfully inoculated into damaged tissue. The fungus is, therefore, considered to be a saprophyte or at the most a weak parasite. *Leptosphaeria* perithecia had previously been found on the host, but on this occasion the ascospores were not grown and the material was discarded. Further perithecial material from young oil-palm seedlings has since been collected by Mrs E. Harris in Northern Nigeria (I.M.I. 73664).

CULTURAL CHARACTERS

The following is an account of the cultural work carried out at the Commonwealth Mycological Institute. A single ascospore culture received from the West African Institute for Oil Palm Research was found on arrival to be producing *Pestalotiopsis* conidia. Subcultures were made on to a variety of agar media and on to sterilized lupin stems in test-tubes. The latter were the more successful in the subsequent production of perithecia. On lupin stems numerous acervuli of the *Pestalotiopsis* produced mature conidia after 3 weeks. These were followed by perithecia which formed asci with mature spores 10–12 weeks after inoculation of the subculture.

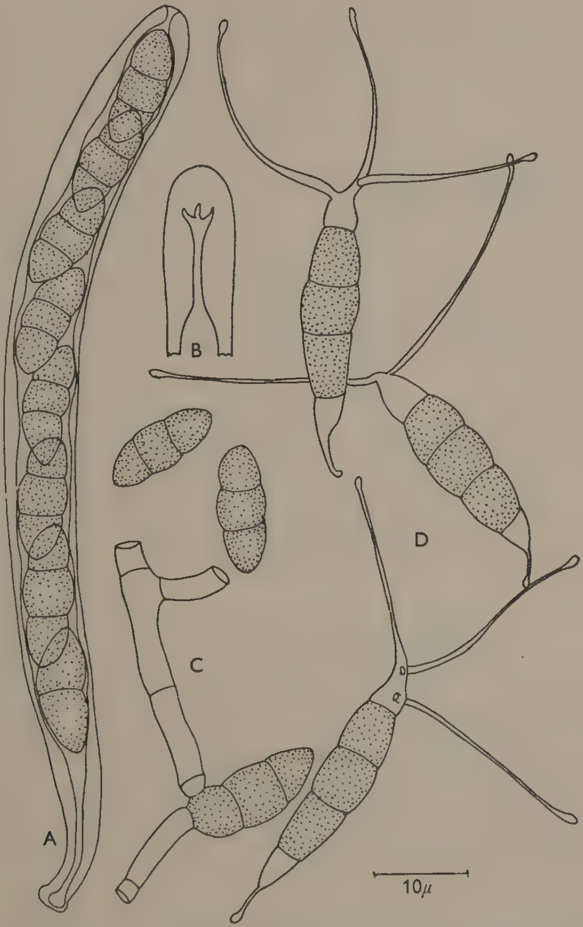

Fig. 1. *Leptosphaeria elaeidis*. A, mature ascus. B, tip of young ascus. C, ascospores, lower one germinating. D, three conidia.

Six single ascospore isolations were made from one of these perithecia and placed on to 2% potato dextrose agar. When these had begun to grow, subcultures were taken and placed on sterile lupin stems. The ascospore isolations on potato dextrose agar formed the *Pestalotiopsis* state only, but the cultures on lupin stems formed first the *Pestalotiopsis* state and, approximately 10 weeks later, perithecia with mature asci and spores.

Ascospores germinate readily on agar, a hyphal germ tube arising from one or both ends of the spore. On potato dextrose agar in tubes the surface is covered after 10 days by a sparse, white mycelium which later turns slightly brown and is accompanied by a darkening of the agar below. The stromatic aggregations, which on agar represent the acervuli, form below this mycelium and their position is marked by the extrusion of the 'black' conidia after about 3 weeks.

On lupin stems the pseudoparenchymatous tissue that forms an acervulus is approximately 1 mm. diam. It forms in the epidermis below the cuticle and the latter is raised as it develops. When this stroma is about 30 μ thick a central longitudinal slit occurs which forms the locule and it is from the lower wall of this locule that the conidia arise. With conidial development, the upper wall is forced upwards and this ruptures the overlying cuticle; finally, the upper wall splits and rolls back exposing the conidial mass.

The perithecia are dispersed or gregarious and arise in the cortex. As they develop they raise and finally rupture the overlying epidermis, thus becoming suberumpent. They are black and 200–250 μ high by 120–150 μ diam. The pseudoparenchymatous wall is 9–16 μ thick. In the region of the ostiole the pseudoparenchymatous tissue separates and parallel hyphae form the ostiolar papillae. Pseudoparaphyses are present between the asci.

Leptosphaeria elaeidis sp.nov.

Perithecia dispersa vel subaggregata, subepidermalia obpyriformia, atrobrunnea, 120–150 μ diam. et 200–250 μ alt. Parietes 9–16 μ crassi. Asci cylindracei, bitunicati, 86–102 × 6–8 μ, 8-spori. Ascosporae monostichae, 12–21 × 4½–6 μ, cylindraceae vel ellipsoideae, brunneae, 2-septatae, constrictae. Paraphysoidibus fibrosis. Conidia instar Pestalotiopsidis clavato-fusiformia, 4-septata, (23)27–34 × 6–7(10 μ); cellulae mediae concolores brunneo-olivaceae (15)19–21 μ; cellula apicalis hyalina, conoidea, 2–3 setulas, spathulatas, 19–24 μ longas gerens; cellula basalis in pedicellum 5–7 μ terminans.

Habitat in foliis Elaeidis guinensis, Nigeria, September 1955. Typus I.M.I. 61175.

Since this paper was written Dr H. J. Hudson has isolated this species from sugar cane in Jamaica (I.M.I. 79262).

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ANOTHER AQUATIC SPORE-TYPE WITH CLAMP CONNEXIONS

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(With Plate 1 and 2 Text-figures)

A branched septate spore-type with clamp connexions is reported from stream scum in Nigeria, West Africa. It consists of a long main axis with usually three laterals. In general structure it agrees with spores of the genus *Tricladium*, in which, however, clamps are absent.

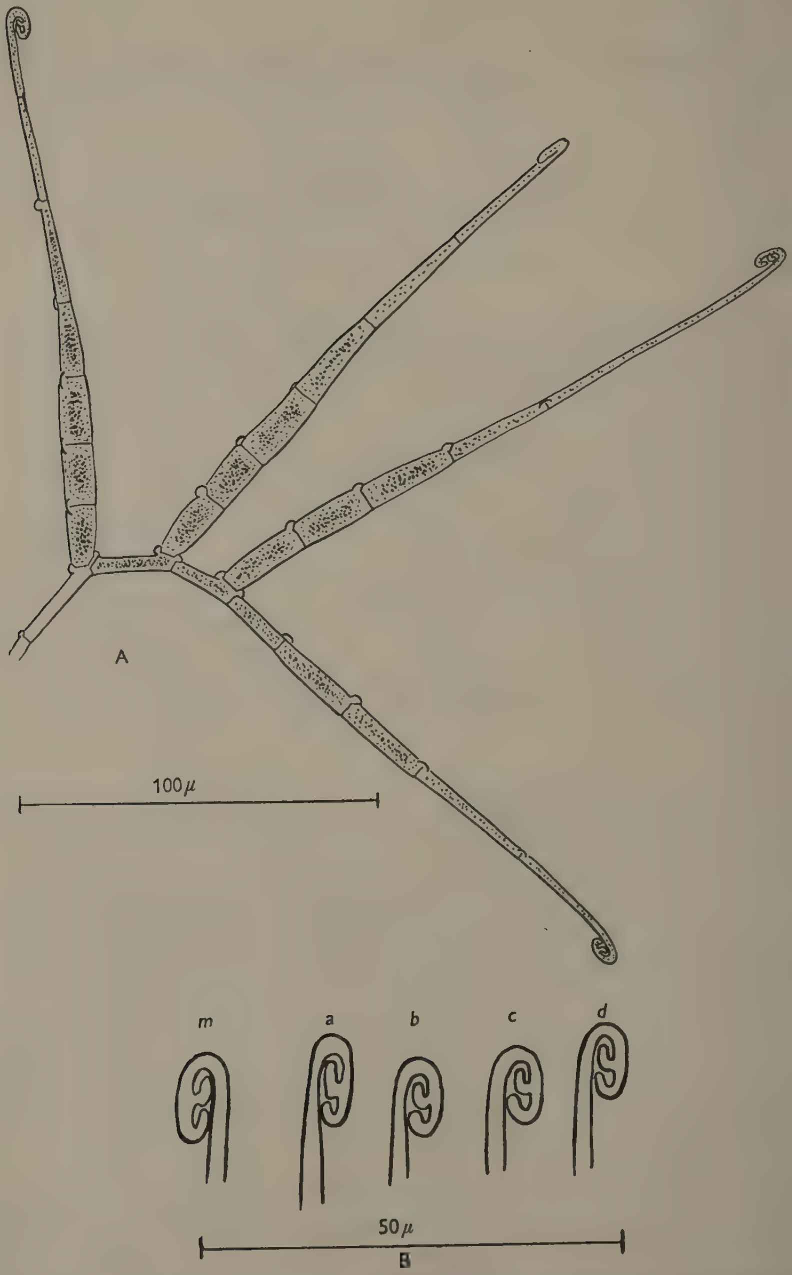
In a recent paper I described (Ingold, 1959) a type of tetra-radiate septate spore with clamp connexions from the scum of a stream in Nigeria. The scum also contained a considerable range of spores of aquatic hyphomycetes including tetra-radiate, multi-radiate and worm-like types.

Recently Mr C. N. Williams sent me a further collection of scum from a stream 15 miles from Ijebu-Ode on the way to Omo. This contained a considerable assortment of spores of aquatic hyphomycetes, including almost all the types already described from Nigeria (Ingold, 1956, 1959), and also a very few examples of the tetra-radiate form with clamps. In addition, however, there were present a considerable number of branched spores of another and distinctive construction, also clamped. These are the subject of this paper.

The spore is a branched, septate structure consisting of a main axis and two to four lateral branches. Of the spores examined the great majority had three laterals. A typical example (Text-fig. 1 A; Pl. 1) and the range of form (Text-fig. 2) are illustrated. The cell walls are very thin and unpigmented. Each cell, except the lowermost on the main axis, which is usually empty, contains unvacuolated protoplasm. Below the basal cell of the spore are the remains of another cell. Clearly spore liberation is not by disarticulation at a cross-wall, but by breakdown of a cell as in *Tetrachaetum* (Ingold, 1942). A clamp connexion can almost always be recognized associated with each cross-wall. Each lateral branch appears to have two basal clamps, but one of these is connected with the lowermost cross-wall of the branch and the other with a wall of the main axis immediately above the origin of the branch.

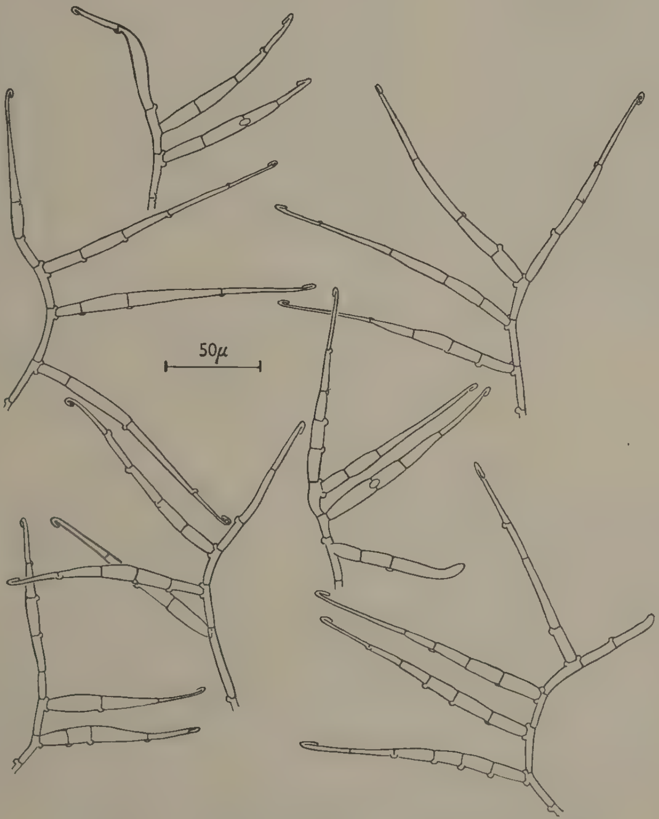
Each of the larger cells in the basal parts of the laterals contains a somewhat elongated central region of denser protoplasm. Within this region, in a lacto-phenol–cotton-blue mount, as a spore takes up the dye, two similar circular bodies stain precociously and these seem to be nuclei, suggesting that the cell is dikaryotic.

The tip of the main axis and of each lateral branch is usually bent over as a hook. This is a complex structure normally with a subsidiary hook (Text-fig. 1 B). These hooks bear only a superficial resemblance to those



Text-fig. 1. A. Typical spore showing cell contents. B. The apices of the main axis (*m*) and the four lateral branches (*a*–*d*) of a single spore.

of the spore of *Ankistrocladium fuscum* described by Perrott (1960), and I should not imagine that they have any special biological significance. Perhaps they represent a rather bizarre distortion of clamp formation associated with the final arrest of growth at the apex of a specialized hypha. Rarely a lateral branch or the main axis is without a hooked apex.



Text-fig. 2. Range in form of spore (cell contents not shown).

In discussing the tetra-radiate type of spore with clamp connexions (Ingold, 1959), I suggested that it consisted essentially of a main axis bent sharply somewhere near its middle and of two laterals arising at the point of flexure. In this respect it would agree in basic structure with the terminal thallospore of *Tetrachaetum*. Similarly in the spore type described here, the agreement is with the terminal thallospore of *Tricladium*. It is tempting to suggest that there may be a whole series of fungi with clamp connexions, and therefore probably basidiomycetes, producing aquatic propagules of forms parallel with those of the aquatic hyphomycetes. A word of caution is, however, necessary. The source of the structures seen in the scum is not known and it is only by inference that they are to be associated with submerged aquatic fungi, although I should be extremely surprised if they were not. It is now urgently desirable that the full life-story of these presumed aquatic basidiomycetes should be investigated.

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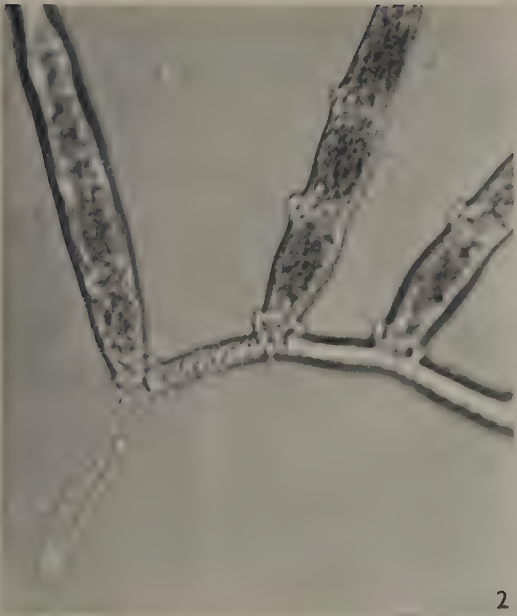
EXPLANATION OF PLATE 1

Branched aquatic spore with clamp-connexions.

Fig. 1. Whole spore. × 400.
Fig. 2. Part of the main axis of the same spore. × 800.
Fig. 3. The apex of one of the branches. × 800.

(*Accepted for publication* 7 *January* 1960)



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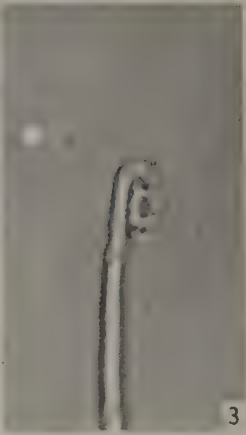

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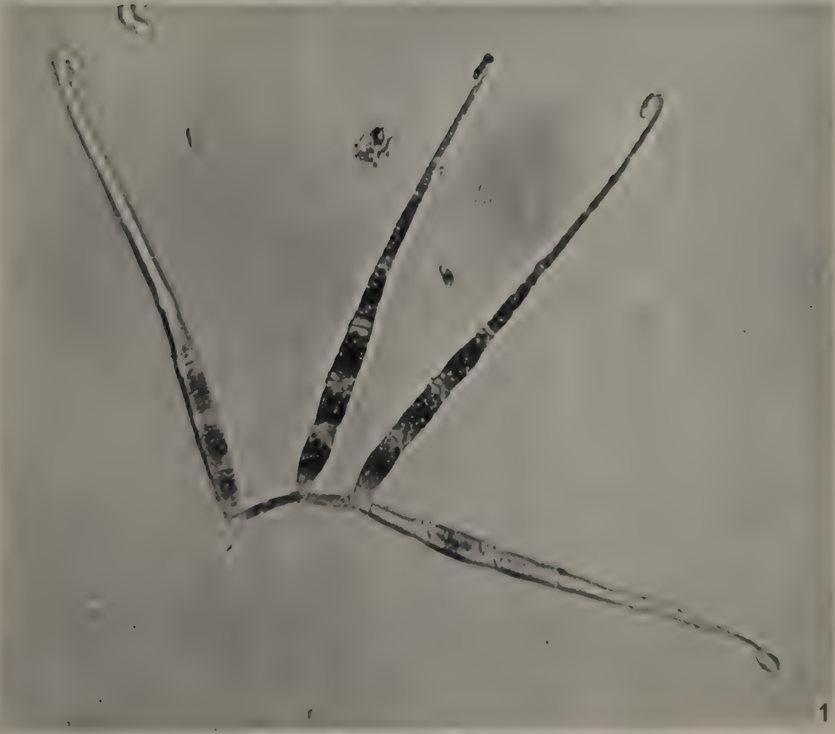

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(*Facing p.* 30)

Trans. Brit. mycol. Soc. **44** (1), 31–41 (1961).

OBSERVATIONS ON THE GENUS *MYROTHECIUM*

III. THE CYLINDRICAL-SPORED SPECIES OF *MYROTHECIUM* KNOWN IN BRITAIN

By N. C. PRESTON

(With Plate 2 and 1 Text-figure)

Since 1943 many additional collections of *Myrothecium* have been examined. *M. carmichaelii* Grev. and *M. roridum* Tode are now considered to be distinct species and a neotype for *M. roridum* Tode is proposed. A key to the cylindrical-spored species is given and their history reviewed.

When Preston (1943) first reviewed the genus *Myrothecium* only some ten living collections were available for comparison with the published exsiccata. He was able, however, to recognise the three classic species which Fries had accepted, viz. *M. inundatum*, *M. roridum* and *M. verrucaria*, each of which has been found in Britain, and he added *M. striatisporum* Preston to the British list (1948). Since then many more specimens have been seen and the purpose of this paper is to explain and, one hopes, to substantiate the resulting modified views now put forward concerning those species which have cylindrical spores.

Generic description of Myrothecium

Sporodochia small (0·1–1·5 mm. diam.), discoid, sessile or with very short stalks, often confluent, black, white rimmed; *spore mass* viscous and green when young, later becoming hard and black; *conidiophores* of branched, septate, hyaline hyphae, their apical cells surmounted by verticils of slenderly clavate, hyaline, finally agglutinated phialides; *spores* cylindrical, ovate or fusoid, continuous, hyaline to olive brown, often guttulate.

Key to the cylindrical-spored species of Myrothecium *mentioned in this paper*

Sporodochia with setae

Setae evanescent, thick-walled, septate, hyaline, about 100–300 μ long, 3–4 μ wide at the base tapering to 2 μ near the bluntly pointed apex. *Phialides* very slenderly clavate, hyaline, 9–22 × 1–2 μ. *Spores* 3–4 × 1–2 μ, cylindric with rounded ends, subhyaline; known only on dried up agarics. *M. inundatum* Tode ex Fr.

Sporodochia without setae

Marginal ring consisting of hyaline, loosely intertwined, contorted, unseptate hyphae with blunt ends; fertile hyphae 3–4 celled, cells *ca.* 30 × 3 μ at the base, 10 × 1·5 μ at the apex. *Phialides* slenderly clavate, 10–12 × 1–2 μ, usually arranged in whorls with 3–7 in each whorl.

1 Spores slender, at least five (usually 7–10) times as long as wide; 2–3 guttulate, subhyaline, black in mass; 8–13 × 1–1·5(rarely 2) μ; originally

on decaying vegetable matter; more recently on dead stems of species of *Thalictrum*, *Eupatorium* and *Iris* *M. carmichaelii* Grev.

2 Spores always less than five times as long as wide, hyaline or subhyaline, black in mass, often biguttulate; 5(rarely 4)–11 × 1–3(usually 2) μ; on moribund and dead stems of a diversity of cultivated plants, very rare on indigenous species in Britain *M. roridum* Tode ex Fr.

The remaining known British species have ellipsoid or fusoid spores, but they should, perhaps, be mentioned here:

M. verrucaria Ditmar:* spores ellipsoid or lemon shaped, truncate, hyaline to subhyaline, black in mass, 6–10 × 2–2·5 μ.

M. striatisporum Preston: spores subfusoid, truncate, subhyaline to smoky olive brown, 7–12 × 2·5–3 μ.

The latter is at once recognizable by the spirally arranged ridges (about 15 in number) which give the epispore a characteristically fluted appearance.

EXSICCATA

(When any particular exsiccatum is referred to in the text the number preceding it here is quoted. The letter in parentheses indicates the herbarium where the specimen is to be found.)

M. INUNDATUM

1 (**K**). 'Myrothecium inundatum Tode, Fries, *S.M.*, III, p. 217' (scripsit J. F. Klotzsch). 'Carm.' (scripsit W. J. Hooker). 'Sporidia cylindrica. An varietatem Myrothecii roridi?' (scripsit M. J. Berkeley). Collected between 1825 and 1830.

2 (**K**). Fuckel's *Fungi Rhenani*, p. 165. 'Myrothecium inundatum Tode. Ad Agaricos putridos, raro. In Sylva Hostrichiensi.' (Cent. 2: publ. 1863.)

3 (**K**). Rabenhorst, *Fungi Europaei*, p. 517. 'Myrothecium inundatum Tode. *Mechl. Schw.* f. 39. Ditm. in Sturm's *D. Fl.* III, T 3. Rabenhorst, *Handbuch*, I, p. 49. Ad Cantharellum cibarium aliosque fungos putridos, autimno....-echorst. leg. Dr T. H. Spree.' (Cent. 6: publ. 1863.)

4 (**K**). Rabenhorst ,*Fungi Europaei*, p. 679. 'Myrothecium inundatum Tode, *Mechl.* Rabenhorst, *Handbuch*, I, p. 49. In agaricis et Russulis putricentibus prope Gratz Austriae leg. G. de Niessl.' (Cent. 7: publ. 1864.)

5 (**K**). 'Myrothecium inundatum in Russulam adustam, Swanage, Nov. 1857' (scripsit M. J. Berkeley). Collected C. E. Broome.

6 (**K**). 'Myrothecium Carmichaelii Grev. Ascot, 31 Oct. 1867' (scripsit M. J. Berkeley). [On an old agaric.]

7 (**K**). 'Myrothecium roridum Tode on Russula adusta; C. B. Plowright, King's Lynn, Aug. 1876.' M. C. Cooke, *Fungi Britannici Exsiccati*, ed. 2, p. 628.

8. 'Myrothecium inundatum Tode on Russula adusta North Wootton, May 1935, T. Petch.' (Herb. N.C.P. XV*a*.)

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* Evidence that the name *Myrothecium verrucaria* must rightly be attributed to Ditmar alone has yet to be considered in a subsequent paper.

9. Myrothecium inundatum Tode on Russula adusta; Wheatfen Broad, Norfolk; E. A. & M. B. Ellis; I.M.I. 5607, 25 May 1946; 8983, 10 June 1946.

M. CARMICHAELII

10 (**E**). 'Myrothecium Carmichaelii, Grev. Appin. Capt. Carmichael.' Type (collected presumably before 1825.)

11. On *Eupatorium cannabinum*; Strumpshaw Marsh, Norfolk (burned in spring of previous year); E. A. Ellis, 3 April 1949. I.M.I. 34763.

12. On *Thalictrum flavum* (dead stems); Smee Hole, Wheatfen Broad, Norfolk; E. A. Ellis, 3 April 1949. I.M.I. 34815.

13. On *Iris pseudacorus* (dead stem); Broad Marsh, Wheatfen Broad, Norfolk; E. A. Ellis, 29 May 1950. I.M.I. 41597.

M. RORIDUM

14 (**L**). *Myrothecium verrucaria* Ditmar folder in Herb. Persoon Herb. Lugd. Bat. 910, 225–287.

'Myrothecium nigrescens.
Myrothecium verrucaria Ditm.
Peziza—Albertini et Schweinitz.
Ex Lusatia miserunt: Albertini et Schweinitz.'

(Perhaps the earliest extant collection of a *Myrothecium* with cylindrical conidia.) Fide auctores: 'In foliis exesissimis subconfectis *cucumerinis*'; collected presumably before May 1804.

15 (**G**). Herbier Berbey Boissier. 'No. 166 Nassau's Flora. Myrothecium roridum Tode; All(ium) sativ(um), in horto meo, Aut. Leopold Fuckel.' [Distributed in Fuckel's *Fungi Rhenani*, p. 166.] (Publ. 1863.)

16 (**K**). 'Fungi North America. Myrothecium roridum Tode, Aiken S.C(arolina). H. W. R(avenal).'

17 (**PAD**). Herb. P. A. Saccardo. 'Myrothecium roridum Tode, Hymenula chartarum Padova.... 76. I.' [P. A. Saccardo; on paper. Jan. 1876. Figured in *Fungi Italici*, Tab. 858; notable as probably the first specimen of a *Myrothecium* from which spore measurements were made and published.]

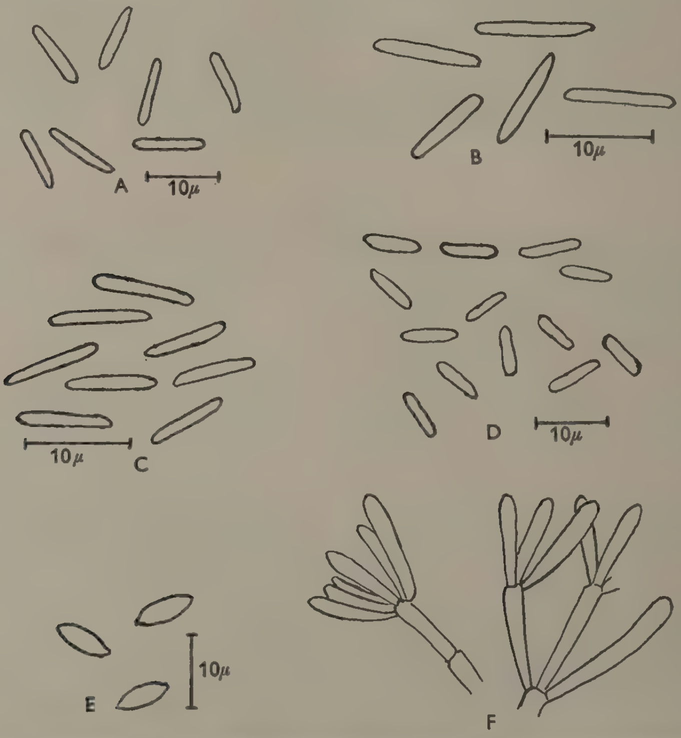
18 (**BM**). 'Myrothecium roridum Tode on dying Violas. Oct. 1920' (scripsit W. B. Grove). [Earliest extant British collection of genuine *Myrothecium roridum*, as opposed to *M. inundatum* and *M. carmichaelii*.]

19. Herb. N.C.P.; isolation no. 63β. *Myrothecium roridum* Tode, from *Viola tricolor* (dying cultivated plant); Wellington, Shropshire, 1934. [The earliest known isolation of *M. roridum* from a British collection; the collection was not preserved.]

20. Herb. N.C.P. Culture No. 6. *Myrothecium roridum* Tode from *Antirrhinum majus* (roots). F. T. Brooks, Aug. 1939.

21. Herb. N.C.P. No. 65. *Myrothecium roridum* Tode on inside of stem of unidentified Umbellifer; Collected J. Webster, 1951, Downton Gorge, Hereford foray, 17 Sept. 1951. [The only known collection of *M. roridum* growing spontaneously on a wild plant in Great Britain.]

In 1790 H. J. Tode proposed the genus *Myrothecium* with the diagnosis: '(Myrothecium). *Fungus cyathiformis*; *cupula superni volvata*; *repletura seminale lentoviscida.*' [Fungus cup-shaped; cups surrounded by a sheath; when filled with spores slowly sticky.] He described five species, *M. inundatum*, *M. roridum* and three others which, according to Fries (1829), belonged to quite other genera. The habitat of *M. roridum* was given as the damp remains of oak and pine branches, that of *M. inundatum* as a dried up agaric.



Text-fig. 1. A. Spores of *M. carmichaelii* from type specimen; B. Spores of *M. carmichaelii* from *Thalictrum flavum* (M.J. *del.*); C. Spores of *M. carmichaelii* from *Eupatorium cannabinum* (M.J. *del.*); D. Spores of *Peziza verrucaria* from exsic. in Herb. Persoon; E. Spores of *M. verrucaria* from exsic. J. B. Ellis; F.N.J. 2769. F. *M. roridum*; Fuckel No. 166 on *Allium*; conidiophores. × 1500 approx.

In 1809 Link emended Tode's generic diagnosis and accepted within the genus only *M. inundatum*. Since Tode had not described the spores, which would have been invisible to him, it seems that Link must have used the peculiar habitat of this species as his means of identifying it. He described it as follows: 'Sporangium figurae indeterminate. Peridium simplex, laxe floccosum tandem in medio evanescens. Sporidia minutissima globosa, prima aetate fluidiuscula, dein compacta.' [Sporangium of irregular shape. Peridium loosely woolly, at length disappearing at the centre. Spores very small globose, at first somewhat fluid then compact.]

Note. The spores are so small that they can appear globose as they rotate under the microscope. They were again described as globose by Ditmar (1813).

Persoon (1818, p. 114) referred to the genus and stated that *M. inundatum* is found only on dried agarics, thus: 'L'éspèce ordinaire est le Myrothecium viride (*M. inundatum*, Sturm. Champ. cah. 1, t. 3.) dont le disque est un verte olivâtre: elles ne vient pas ailleurs que sur les Agarics secs, et particulièrement sur l'Agaricus adustus ou nigricans, Bull....'

Gray (1821) made what seems to be the first traceable reference to *Myrothecium* as a genus belonging to the British fungus flora by including in his list *M. inundatum* with the note 'On rotten fungi, autumn'. This entry of Gray's is perhaps the first occasion on which the name *Myrothecium* was used after 1 Jan. 1821.

About 1824–25 Carmichael collected the earliest extant specimens of *Myrothecium* in Great Britain. Some of these he sent to W. J. Hooker at Glasgow and others to R. K. Greville at Edinburgh.

Greville (Scottish Cryptogamic Flora, 1825) identified one of Carmichael's specimens as a new species, *M. Carmichaelii*, which, he said, differed from the other species known to him in having cylindrical spores. He stated: 'For the addition of this highly curious genus to the British Flora we are indebited to Captain Carmichael whose specimens are abundantly distinct from the other two species which have been described.' Greville refers to the spore shape of the other species thus: 'M. verrucaria Ditm. has ovate sporules. M. inundatum (Tode) has globose ones: the three species have, however, such a strong resemblance to each other, that it is necessary to examine them under the microscope, in order to ascertain their distinctive characters.'

Greville's description of his new species was: 'Myrothecium Carmichaelii. Carmichael's Myrothecium. Specific character. Myrothecium Carmichaelii; peridio polymorpho, candido; sporulis viridibus nigrescentibus cylindricis. M. peridium irregular in form, white; sporules green, becoming blackish, cylindrical. Hab. on masses of subputrescent vegetable substances. Appin, Captain Carmichael, viz. *Sporangia* of various forms, 1–3 lines in length or breadth, rarely exactly circular, often irregularly sinuose, white depressed. *Peridium* formed of an interwoven mass of fine filaments, which gradually give way, and at length altogether disappear towards the centre, leaving the mass of sporules exposed. *Sporules* forming a semifluid pulp in the young state, in the whole genus, at length compact but not pulverulent. In the present species they are exactly cylindrical.' He also records seeing what appear to have been the intertwining fertile hyphae and the phialides which make up the hymenial layer, thus: 'I have sometimes thought I perceived certain erect hyphae or cells throughout the whole central substance, within which the sporules are situated; this, however, remains to be confirmed.'

Fries (1829) referred *M. carmichaelii* to *M. roridum* and, along with *M. inundatum* he also accepted *M. verrucaria.* He distinguished these three species as follows: *M. roridum* disk swollen, spores cylindric; *M. verrucaria* disk flattened, spores ovate; *M. inundatum*, disk flat, spores globose.

Note. Close examination of the three classic species has shown that the shape of the 'disk' is no sound criterion for their precise identification.

Within the period 1820–30, M. Klotzsch was assisting with the arrangement of Hooker's herbarium and there noticed that one of Carmichael's specimens of *M. inundatum* on a dried agaric (exsic. 1), had cylindrical, not globose, spores. This fact was referred to later by Berkeley (1836). He examined the specimen of *M. inundatum* previously seen by Klotzsch and confirmed that its spores were indeed cylindrical, annotating its label to this effect (exsic. 1). For this very reason, Berkeley regarded the fungus which his forerunners had accepted as *M. inundatum* as but a variety of *M. roridum*, to which species he had assigned all cylindrical-spored specimens of *Myrothecium*. It was thus that the name *M. roridum* was introduced into British fungology.

Berkeley wrote '1. *M. roridum* Tode, (Spangled Myrothecium), disk turgid, sporidia cylindric. Tode, Fung. mech. f. 38. Fr. Syst. Myc. v. 3, p. 217. M. Carmichaelii, Grev. Sc. Crypt. Fl. 140. On rotting plants, dried agarics, etc., Appin. Capt. Carmichael. The specimens on dried agarics, though agreeing at first sight with *M. inundatum*, have cylindric not globose *sporidia*. This fact was noticed by M. Klotzsch when inspecting Dr Hooker's herbarium and my own observations are in accordance with his. This is one among many instances of always have recourse to the microscope for the correct determination of the more minute fungi.'

Note. The first occasion in which the practice of measuring spores was introduced into British mycology was in Berkeley & Broome's *Notices of British Fungi*, No. 690 (1854). Accordingly Berkeley did not notice the marked difference in size of the spores of *M. inundatum* and of *M. carmichaelii*.

Exsic. No. 1, referred to in the preceding paragraphs, is notable as being the earliest extant specimen of *M. inundatum* collected in Britain and also the first in which the spores were recognized as being cylindrical. The latter fact was not further confirmed until Höhnel (1905) pronounced them to be oblong rod-shaped and not globose. Höhnel also recognized the long, white, evanescent setae, present in young specimens.

Saccardo (1880) identified *Myrothecium roridum* on wet paper and rotting stems of *Malva* and gave this description. '*M. roridum*, sporodochia, plana, atra, albomarginata, conidia cylindrica, utrinque obtusa 8–10 × 2 (raro 14 × 2) μ, 2 guttulata, dilute olivacea, basidiis filiformibus 30 × 2 μ sulffata.' [*M. roridum*, sporodochia flat, black with a white margin, conidia cylindrical with blunt ends, 8–10 × 2 (rarely 14 × 2) μ, biguttulate, pale olivaceous, supported on filiform basidia 30 × 2 μ].

Note. It was not until after 1908 that Saccardo (1913) began to use the term *conidiophore*, instead of *basidium* to denote the spore-bearing cell of *Myrothecium*.

A preparation from W. B. Groves's collection (exsic. 18) has been examined and the identity with *M. roridum* confirmed. All previous British records of so-called *M. roridum* were, in fact, based on *M. inundatum* on dried agarics or on the type collection of *M. carmichaelii*.

M. roridum was next recorded in 1932 when it was found by Preston in a garden in Shropshire, again associated with dying violas. It was then identified by Miss E. M. Wakefield (exsic. 19).

During the period 1936–43, Brooks (1945) found *M. roridum* at Cambridge, where it was apparently parasitic upon the roots of *Antirrhinum majus*, *Lupinus ornatus*, and *Solanum tuberosum*; he isolated the fungus from these plants and sent cultures to Preston (exsic. 20).

The finding by E. A. Ellis of *M. carmichaelii* in 1949 on *Eupatorium cannabinum* and *Thalictrum flavum* (dead stems) in the Norfolk Broads (exsic. 11 and 12) was the first record in Great Britain or elsewhere since the first description. It was found again in 1950 (exsic. 13).

In 1951 we have the first instance, and so far the only one, of *M. roridum* on an uncultivated plant, growing in its natural habitat in Great Britain. This specimen was collected by Dr J. Webster from the hollow stem of an unidentified umbelliferous plant (exsic. 21). The fungus appears to be relatively rare in this country. On the other hand, it seems to be quite common in tropical and subtropical regions, frequently on leaf spots, as reference to Table 1 will show.

The few collections of *M. carmichaelii* which I have seen are all of British origin and were found on uncultivated plants or, as for the type, on plant debris.

When redescribing *M. roridum* as one of the three classic species Preston (1943) figured the spores of the type, *M. carmichaelii*, and also those of three other specimens which had been referred to *M. roridum* (exsic. 19 and 20). Although his figures show clearly the difference in length–width ratio between the spores of the type and those of the other three specimens, Preston decided to follow Fries and accept *M. carmichaelii* as the type for *M. roridum* since, at that time, there seemed insufficient evidence to justify a contrary view.

From the additional material which has become available since 1943 it has become evident that the spores of *M. carmichaelii* are consistently longer and more slender than those of *M. roridum* (see Tables 1 and 2). This difference is sufficiently clear to be appreciated at sight and the two fungi are thus readily distinguishable.

Note. Under modern conditions a single sporodochium of *M. roridum* can be relied upon to produce an immense number of sporodochia in artificial culture. This unfortunately does not apply to *M. carmichaelii* which remains persistently sterile on artificial media.

The facts set out in the foregoing paragraphs lead one to conclude that Fries's acceptance of *M. carmichaelii* as the type for *M. roridum* is no longer tenable and that, in the light of our present knowledge, these two should properly be regarded as distinct species.

The name *Myrothecium roridum* has been in existence for more than 150 years; it is being applied to numbers of collections and cultures over the whole world. For so long as *M. carmichaelii* was treated as a synonym of *M. roridum*, the type of *M. carmichaelii* could stand duty for *M. roridum* also, but now that the two are separated *M. roridum* is left without a type and a neotype for it must be sought.

For this neotype three possible specimens exist, these are:

(1) Specimen from an authentic collection of '*Peziza verrucaria*' A. & S. in Herb. Persoon (exsic. 14). Only a single stroma could be found on this material; the conidia are cylindrical.

Note. The name *Myrothecium verrucaria* Ditmar is reserved for a species with ovate, not cylindrical conidia.

(2) Fuckel's collection on *Allium sativum*, No. 166. In Herbier Berbey Bossier (exsic. 15). Through the courtesy of the Director of the Conservatoire botanique, Geneva, I have been able to examine this material. Sporodochia are present and the fungus can be identified with certainty

Table 1. *Collections referred to* Myrothecium roridum

(In order of average length of spores)

Herb. N.C.P.	Herb. I.M.I. no.	Spore range (*μ*)	Average (*μ*)	Host	Country of origin
	1520 as *M.* sp.	4–6 × 1·5–2	5 × 1·8	*Rinorea microdon*	Tanganyika
29	36519	5–6 × 1–1·5	5·4 × 1·2	*Hibiscus esculentus*	Malaya
20	34468	5–6 × 2	5·5 × 2	*Vigna sinensis*	Malaya
32	35585	5–6 × 1·5–2	5·6 × 1·9	*Glycine max*	Malaya
	24912	5–6 × 1·5–2	5·6 × 1·9	*V. sinensis*	Malaya
33*a*	37806	5–7 × 2–2·5	5·8 × 2·2	*Luffa acutangula*	Malaya
	1509	5–7 × 1·5–2	5·9 × 1·9	*Phaseolus mungo*	Tanganyika
61		5·5–6·5 × 2	6 × 2	*Lactuca sativa*	England
	1510	5–7 × 2	6 × 2	*P. vulgaris*	Sierra Leone
33	35614	6–7 × 2	6 × 2	*Luffa acutangula*	Malaya
	1512	5·5–7 × 2	6 × 2	*Canavalia ensiformis*	Sierra Leone
	1513	5–7 × 2–2·5	6·2 × 2·2		
	1506	6–7 × 2–3	6·2 × 2·2	*P. adenanthus*	Sierra Leone
9	37900	5–7 × 2	6·2 × 2	*Lupinus ornatus*	England
65	62420, 47245	5–7 × 1·5–2	6·1 × 2	Umbelliferae sp.	England
	13849	5–7 × 1·5–2	6·3 × 1·6	*V. sinensis*	India
	35611	5–8 × 2	6·1 × 2	*Psophocarpus tetragonolobus*	Malaya
	1511	6–7 × 2	6·3 × 2·1	*Phaseolus lunatus*	Sierra Leone
	1503	5·5–7 × 1·5–2	6·3 × 1·9	*Asystasia sp.*	Sierra Leone
849	1500	6–7 × 2	6·4 × 2	*Trichosanthes anguina*	Sierra Leone
	1498	6–7 × 2	6·3 × 2		
	1499	6–7 × 2	6·4 × 2	*Luffa acutangula*	
	1501	6–8 × 1·5–2	6·5 × 1·9	*Impatiens sultani*	
10	37901	5–7 × 1·5–2	6·5 × 2	*Solanum tuberosum*	England
	1516	5–8 × 1·5–2	6·5 × 2	*Sida rhombifolia*	Sierra Leone
	1519	6–7 × 2	6·5 × 2	*R. microdon*	Sierra Leone
	1507	6–7·5 × 1·5–2	6·6 × 2	*P. lunatus*	Nigeria
31	36408	5–7·5 × 1·5–2	6·6 × 1·9	*Musa sapientum*	Sierra Leone
54	44589	5·5–7·5 × 2–2·5	6·6 × 2·1	*Ficus* sp.	Ghana
57		5–8 × 1·5–2	6·4 × 1·9	*Viola tricolour*	England
59		5·5–7·5 × 1·5–2·5	6·6 × 2		
97 L. Fuckel No. 166 (exsic. 15)		6–8 × 2	6·5 × 2	*Allium sativum*	Germany
21	34469	6–7·5 × 2	6·7 × 2	*Solanum melongena*	Malaya
	24909 (*c*)	6–7 × 1·5–2	6·7–1·9	*Carica papaya*	Malaya
58		6–8 × 1·5–2	6·8 × 2	*Viola tricolor* (isolated 1934)	England
64	70234	6–8 × 1·5–3	6·9 × 2		
64*a*		6–8 × 2	6·9 × 2		
63*β*	62421	5·2–8 × 2–2·5	6·9 × 2		
1	1502	6–8 × 1–2	6·9 × 1·7	*Asclepias curassavica*	Sierra Leone
25	36559	6–8 × 1·5–2	6·9 × 1·9	*Justicia flava*	W. Africa
	1515	7–8 × 1·5–2	7·1 × 1·9	*Hibiscus esculentus*	Tanganyika
92 P. Saccardo Jan. 1876 (exsic. 17)	69801	5·4–9 × *c.* 2	7·1 × *c.* 2	Paper ('*charta putri*')	Italy
4	1508	5–8 × 1·5–2	7·2 × 2	*Dolichos lablab*	Ghana
	1504	6–8 × 1–2	7·2 × 1·9	*P. adenanthus*	Sierra Leone

Table 1 (*cont.*)

Herb. N.C.P.	Herb. I.M.I. no.	Spore range (μ)	Average (μ)	Host	Country of origin
40*a*		6–8 × 1·5–2	7·3 × 1·7	Fen soil (isol.)	England
53	70238	6·2–7·6 × 2	7·3 × 2	*Pisum sativum*	England
	33307	6·5–8 × 1·5–2	7·3 × 1·7	*Lycopersicon esculentum*	China
849		5·5–9 × 2	7·4 × 2	*T. anguina*	W. Africa
37		5·5–8 × 1·5–2	7·4 × 2	*L. esculentum*	England
6	37899	6–9 × 2–2·5	7·5 × 2·2	*Antirrhinum majus*	England
26		6·0–8·5 × 1·5–2	7·6 × 1·9	*L. esculentum*	England
13*a*		7·5–9 × 1·5–2	8·1 × 2		
50		7·6–9·5 × 1·9–2·9	8·2 × 2·2		
62	70237	7–9 × 2	8·1 × 2		
74		7–10 × 2–2·5	8·2 × 2	*V. odorata* (cultivated)	England
880	1505	6–11 × 2–3	8·3 × 2·6	*Dolichos lablab*	Sierra Leone
12	1521	8–10 × 1·5–2·5	8·7 × 2	*V. odorata*	Tanganyika
23	70239	8–10 × 1·5–2·5	8·7 × 2	*L. esculentum*	England
38		7·5–10 × 2	8·9 × 2		
937	1514	6·5–10·4 × 1·5–2·5	9·4 × 2	*Hibiscus sp.*	Sierra Leone
24		8–10·5 × 2–3	9·3 × 2·4	*L. esculentum*	England

Table 2. *Collections referred to* M. carmichaelii

(All from Great Britain)

Herb. N.C.P.	Herb. I.M.I. no.	Spore range (μ)	Average (μ)	Host
—	44746	8–10 × 1–2	9·5 × 1·3	Decaying vegetable matter
Exsic. 11.	Type in Herb. Hort. Bot. Edinburgh.			
22	34815	8–13 × 1–2	11 × 1·3	*Thalictrum flavum*
22*a*	34763	8–10 × 1	8·7 × 1	*Eupatorium cannabinum*
89	41597	8·1–10·8 × 0·9–1·5	9·8 × 1·3	*Iris pseudacorus*

as *M. roridum* as maintained in this paper. Its spores are rod-like with rounded ends, averaging 6·5 × 2 μ. In the same packet is a distinct *Myrothecium*, with hyaline setae, on *Orobanche rubens*; this is a species unknown to me.

Fuckel did not describe either of these specimens, but he did, however, record (1870, p. 364) finding *M. roridum* on the outer scales of onion from his garden and also on the stem of *Orobanche rubens*. The *M. roridum* on *Allium* was distributed by him as his 'Fungi Rhenani No. 166', but, so far as I can determine, no one has found sporodochia on these published specimens; there appears to be none on the exsiccatum in Herb. R.B.G., Kew.

(3) The specimen on decomposing paper ('*charta putri*') in the Saccardo Herbarium at Padua (exsic. 17). This specimen can be identified as *M. roridum*. Although the script accompanying it is not clear, it would seem to be that figured by Saccardo (1881, No. 585). His description (1880) has been quoted above. The spores, when remeasured, were 5·7–9·5 × 2 μ. I do not know from what specimens Saccardo recorded spores measuring 'raro 14 × 2 μ'.

Of these three specimens, (2) appears most appropriate to our purpose and I therefore propose Fuckel's Fungi Rhenani No. 166, on *Allium sativum* as a so-called neotype for *M. roridum* Tode ex Fries.

Spore measurements for this species (Table 1), as maintained in this paper, extend over a remarkably wide range, within which, and more especially among the tropical specimens, one might perhaps discover the species *M. advena* Sacc. described by Saccardo (1908) as having spores 6–7 × 2 μ and as occurring on leaf spots. I have not seen the type specimen of this species and do not propose to take up this name at present.

When beginning this review I was aware that *M. roridum* frequently occurs on leaf spots on tropical plants, though I have never found it myself occurring thus in Britain. Early experiments (Preston, 1936), however, had shown that spots were produced on the undetached leaves of healthy growing violas when inoculated with a spore suspension of *M. roridum* and I have never attributed any particular significance to this fact.

A point of real interest, with regard to Saccardo's description of *M. advena*, is that between 1908, when his first description was published, and its appearance in the Sylloge (1913), he had adopted the term *conidiophore* ('*conidiophoris fasiculatis*') to describe the spore-bearing cell in place of *basidium* ('*basidiis fasiculatis*') which he had used hitherto for that structure (cf. his description of *M. roridum* in 1880). His figures also show that when using the term conidiophore he was referring to the phialide itself as distinct from the filament on which it was carried.

When first reviewing the genus in 1943 I was unaware of the facts just mentioned but I have, throughout, continued to regard the conidiophores of *Myrothecium* as being made up of the branched, septate, fertile hyphae of which the phialides are a part, i.e. their terminal spore-bearing cells. The term conidiophore is so used throughout this paper.

It is a pleasure to acknowledge here the valuable assistance received from many quarters during the compilation of this paper. I am greatly indebted to the Directors of the following institutions for making available to me critical specimens from the herbaria in their charge: Orto Botanico, Padua; Herbarium, Jardins botanique, Geneva; Rijksherbarium, Leiden; Herbarium, Royal Botanic Gardens, Kew; Commonwealth Mycological Institute, Kew.

To my friend Mr E. W. Mason, formerly of Commonwealth Mycological Institute, I owe a special debt. Without his patient guidance through the mazes of nomenclature this paper would never have emerged therefrom. That he has, moreover, contributed very largely to its actual compilation will at once be clear to all British workers in this field.

I wish to thank Mr W. E. Perry who took all the photographs. I am equally grateful also to Mrs M. Jones for making the line drawings indicated by her initials and also to Miss J. Hickman for so kindly checking my references to all those specimens listed in the tables under Herb. I.M.I. numbers.

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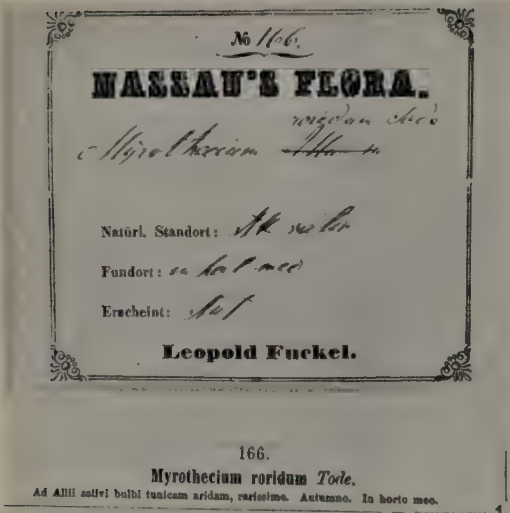
Albertini, J. B. & Schweinitz, L. D. (1805). *Conspectus fungorum in Lusatiae superioris agro Niskensi crescentium*, p. 340, table 8, fig. 46. Described *Peziza verrucaria* on leaves of *Cucumis* and suggested that it was a *Myrothecium.*

Berkeley, M. J. (1836). Fungi. *Smith's English flora* 5, **2**, p. 323. Introduced the name *Myrothecium roridum* into British mycology on two collections both by Capt. Carmichael of Appin; one was the type collection of *M. carmichaelii* and the other was *M. inundatum* on an agaric.


№ 166.

NASSAU'S FLORA.

roridum Tode
Myrothecium ~~Allii~~ m.

Natürl. Standort: All. sativ.

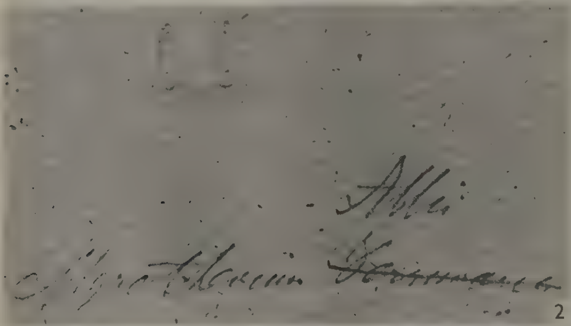
Fundort: in hort. meo

Erscheint: Aut.

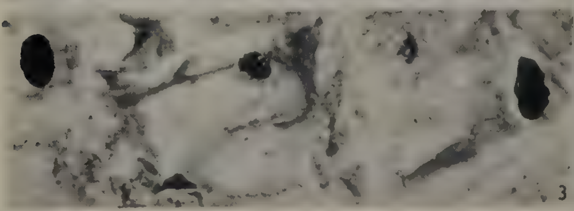
Leopold Fuckel.

166.
Myrothecium roridum *Tode.*
Ad Allii sativi bulbi tunicam aridam, rarissime. Autumno. In horto meo.

1


Allii
Myrothecium [illegible]

2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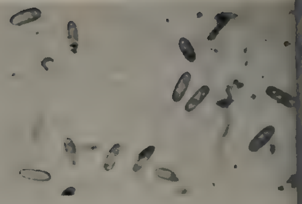

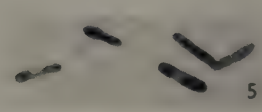
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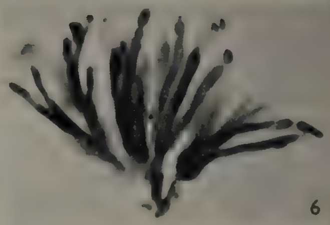
6

7

8

Berkeley, M. J. & Broome, C. E. (1854). Notices of British Fungi, nos. 662–739. *Ann. Mag. nat. Hist.*, ser. 2, **13**, 396–407. Apparently introduced, for the first time, into British mycology the practice of publishing spore measurements of micro-fungi.

Brooks, F. T. (1945). Notes on the pathogenicity of *Myrothecium roridum* Tode ex Fr. *Trans. Brit. mycol. Soc.* **27**, 155–157.

Ditmar, L. P. F. (1813). Pilze. *Sturm's Deutschlands flora*, Abt. 3, **1**, 5–8. Described *M. inundatum* on broken agarics (T. 3) and *M. verrucaria* on *Lapsana communis* (T. 4) and judged that the latter might be identifiable with *Peziza verrucaria* A. & S., after microscopical examination.

Fries, E. M. (1829). *Systema Mycologicum*, **3**, 1, 216–218. Accepted *M. carmichaelii* as identifiable with *M. roridum.*

Fuckel, L. (1870). *Symbolae Mycologicae*, p. 364. Recorded *M. roridum* on the outer scales of *Allium sativum* and on stems of *Orobanche rubens*. The specimen on *Allium*, preserved in his herbarium, is taken, in this paper, as a so-called neotype for *M. roridum* Tode.

Gray, S. F. (1821). *A natural arrangement of British plants.* I, p. 569. Recorded *M. inundatum* for Britain on rotten fungi. Perhaps the first time the generic name was used after 1 January 1821.

Link, H. F. (1809). Observationes in Ordines plantarum naturales. *Diss. Mag. Ges. naturf. Freunde Berlin*, **3**, 1, p. 23. Restricted *Myrothecium* to *M. inundatum* and excluded Tode's four other species.

Persoon, G. H. (1818). *Traité sur les Champignons comestibles*, p. 114. Introduced a superfluous name, *M. nigrescens* Pers. for 'the common Myrothecium'; it survives only on a label in his herbarium.

Preston, N. C. (1936). The parasitism of *Myrothecium roridum* Tode. *Trans. Brit. mycol. Soc.* **20**, 242–251.

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Saccardo, P. A. (April 1880). *Michelia*, **2**, 175.

Saccardo, P. A. (1881). *Fungi Italici autographice delineati.* Tab. 858–860. Figures and gives measurements of *M. roridum, M. verrucaria*, and *M. inundatum.*

Saccardo, P. A. (Oct. 1908). *Ann. Mycol., Berlin*, **6**, 560. Described and figured *M. advena* Sacc.

Saccardo, P. A. (1913). *Sylloge Fungorum*, **32**, 1493. In compiling the diagnosis of *M. advena* he substituted, for the first time, the term '*conidiophoris*' for '*basidiis*'; i.e. for '*cellula fertilis*' but not for '*hypha fertilis*'.

Taubenhaus, J. F. J. (1935). On a black crown rot of greenhouse snapdragons caused by *Myrothecium roridum* Tode. *Phytopathology*, **25**, 969.

Tode, H. J. (1790). *Fungi Mecklenburgenses selecti*, **1**, 25–28. Introduced the genus *Myrothecium* with five species: *M. roridum, M. inundatum, M. hispidum* and *M. dubium.* He placed it next to *Tympanis.*

EXPLANATION OF PLATE 2

Fig. 1. Label attached to Fuckel's collection of *M. roridum*, No. 166 in Herb. Berbey Bossier.

Fig. 2. Fuckel, No. 166. Pencilled inscription and sketch on scrap of paper enfolding fragments of *Allium* bearing sporodochia of *M. roridum.*

Fig. 3. Fuckel, No. 166. Sporodochia on *Allium.* × 20.

Fig. 4. Fuckel, No. 166. Spores from sporodochium on *Allium.* × 1000.

Fig. 5. *M. roridum* on '*charta putri*' in Herb. Saccardo. Spores. × 1500 (approx.).

Fig. 6. *M. roridum* on '*charta putri*' in Herb. Saccardo. Group of phialides with developing spores. × 1500 (approx.).

Fig. 7. *Peziza verrucaria* Albertini & Schweinitz in Herb. Persoon. Spores. × 1200.

Fig. 8. *P. verrucaria* in Herb. Persoon. Fragment of sporodochium with conidiophores and spores. × 1200 (approx.).

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SOME NEW AND INTERESTING SPECIES OF MICRO-FUNGI. II

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(With Plates 3 and 4)

The following are described as new species: *Penicillium kewense*, culture contaminant; *P. kojigenum* from soil, Castle Douglas and Dumfries; *P. madriti*, from garden soils, Madrid; *Aspergillus parvulus*, from forest soil, N. America, and *Chaetomium fusisporum* G. Smith, from soil, Adelaide, Australia, also *A. restrictus* G. Smith mut. *eborinus* mut.nov. spontaneous mutant. *Eladia* gen.nov., type *E. saccula* (Dale) comb.nov., is based on *Penicillium sacculum* Dale. *Mucor vesiculosus* G. Smith (1957) is regarded as synonym of *Gongronella butleri* (Lendner) Peyronel & Dal Vesco.

Penicillium kewense sp.nov. (Pl. 3, figs. 1, 2)

Coloniae in agaro Czapekii vel aliis substratis sublente crescentes, ochraceae, granulosae paene tote ex peritheciis compositae, guttulis rubris numerosis, reverso badio cum agaro subcolorato; perithecia irregulariter globosa vel ovoidea, sclerotiis similia, ex centro lente maturantia, 190–280 μ diam.; asci primum puriformes dein ovoidei, in catenis, octospori, 9–10 × 6–7 μ; sporidia ovoidea, verrucosa, cum cristis duobus equatorialibus late separatis, 4·2–5 × 2·3–2·5 μ; penicilli asymmetrici, compacti, 3-4-verticillati; conidiophora laevia, longa, plerumque 4 μ diam.; metulae 10 × 3–3·5 μ, apice subdilato; phialides 9·5–10 × 2–2·2 μ; conidia plerumque pyriformia, subasperula, 2·8–3·0 × 2·3–2·5 μ, in catenis confertis, subcolumnaribus.

Fungus in culto Scopulariopsis brevicaulis inventus. Typus L.S.H.T.M. BB 400.

Colonies on most of the usual culture media similar, spreading somewhat slowly, consisting almost entirely of perithecia, showing a faintly bluish zone of conidial fructifications only in very young cultures, avellaneous (Ridgway Pl. XL), with numerous reddish drops during the growing period; reverse deep reddish brown with the colour diffusing somewhat into the agar; perithecia 190–280 μ diam., irregularly globose to ovoid, at first sclerotium-like, ripening very slowly from the centre outwards, producing ripe spores in 4–5 weeks; asci pear-shaped when young, becoming ovoid when fully ripe, formed in chains, 8-spored, 9–10 × 6–7 μ; ascospores broadly ovoid with two well-separated equatorial frills, and with the curved surfaces coarsely roughened, 4·2–5 × 3–3·2 μ; penicilli asymmetric, compact, with sometimes three stages, sometimes four stages of branching, when 4-verticillate usually with ramuli and metulae in the same verticil, commonly with 2–3 rami; conidiophores long, smooth, mostly 4 μ diam; rami 20–22 × 3–4 μ; metulae 10 × 3–3·5 μ, slightly swollen at apex; phialides 9·5–10 × 2–2·2 μ; conidia mostly somewhat pear-shaped (appearing globose or subglobose when seen end on), very slightly roughened, 2·8–3 × 2·3–2·5 μ, in chains forming compact masses, almost columnar.

In April 1959 I received from C.M.I., Kew, a culture stated to be a *Scopulariopsis* with cleistothecia, that is with a perfect state quite different from the usual perfect states of *Scopulariopsis* species, which belong in *Microascus* Zukal. The culture was a transfer which had been prepared from a stock culture, stored under oil, of *S. brevicaulis* NRRL 1096, originally received by C.M.I. from this laboratory and obtained by me from the NRRL Laboratories, Peoria, Illinois.

The C.M.I. culture was plated out and subcultures were taken, working under a binocular microscope, from typical *Scopulariopsis* heads and from young, well-separated perithecia. The culture was thus separated into two pure lines, one being *S. brevicaulis*, identical with NRRL 1096 as maintained in this laboratory, the other being the species described here as *Penicillium kewense*, Cat. No. BB400.

In an attempt to identify the mould with one of the known species, the sclerotium-like nature of the young perithecia naturally led to the Carpenteles section of the Divaricata. However, the ascospores were found to be quite different from those of any of the three known species of this section. In addition, the penicillate fructifications are not divaricate but typically asymmetric, this being the first recorded instance of the production of perithecia by a member of the Asymmetrica. In general appearance of cultures *P. kewense* is similar to *P. baarnense* van Beyma. It differs from the latter in producing more pigment in reverse of colonies, also in having ascospores which are smaller and with different ornamentation. In *P. baarnense*, according to Raper & Thom (1949), the ascospores are slightly rough and have two very close frills with virtually no furrow between. According to van Beyma the ascospores are smooth and have a single frill. In contrast, the ascospores of *P. kewense* have a well-marked furrow with two widely separated frills, and are coarsely roughened. The two differ also very markedly in their conidial fructifications.

Penicillium kojigenum sp.nov. (Pl. 3, fig. 3)

Coloniae in agaro Czapekii sublente crescentes, velutinae, viridi-coeruleae dein griseo-viridae, cum margine alba, subfimbriata, reverso incolorato; conidiophora longa, laevia, 2·5–3 μ diam.; penicilli irregulariter 3-4-verticillati; rami 20–30 μ longi; metulae 11–13 × 2·5–3 μ; phialides 10 × 2–2·2 μ; conidia globosa vel subglobosa, asperula, 2·2–2·6 μ diam., in catenis longis diversis dein implicatis.

Typus in solo, Gelston, Castle Douglas, inventus. Typus L.S.H.T.M. BB394.

On Czapek agar grows moderately well, velvety, at first pale bluish green, then developing dark grey-green shades from the centre outwards; margin somewhat fimbriate, white; reverse uncoloured; conidiophores long, smooth, 2·5–3 μ diam., showing a slight tendency to fasciculation; penicilli mostly 3 times verticillate, with 2–3 rami 20–30 μ long, but sometimes with metulae and phialides in the same verticil, hence partially 4-verticillate, sometimes with a secondary branch from a lower node, occasionally with one of the rami bearing phialides directly; metulae 11–13 × 2·5–3 μ; phialides 10 × 2–2·2 μ; conidia globose to subglobose, rough, 2·2–2·6 μ diam., in chains which are at first divergent then tangled.

The type strain, L.S.H.T.M. Cat. No. BB394, was isolated by Mrs S.

Marcus in 1952 from roadside soil, Gelston, Castle Douglas. Another strain, Cat. No. S.M. 851, isolated from soil of pine wood, Dumfries, is closely similar in appearance and reactions. This species is unusual in that it gives good yields of kojic acid when grown on synthetic culture media containing glucose. This substance is a characteristic metabolic product of the *Aspergillus flavus* and *A. tamarii* series. Up to the present only one strain of *Penicillium* has been reported as producing kojic acid (Birkinshaw, Charles, Lilly & Raistrick, 1931). This, Cat. No. Ad116, was obtained from the C.B.S., Baarn, in 1929 as *P. lanosum* Westling. It was sent to Thom, who stated that it fits *P. daleae* Zaleski. However, my own examination (at a much later date) showed that it does not agree with Thom's own description of *P. daleae*, but fits very well with his diagnosis of *P. lanosum*, and still better with Westling's own diagnosis. It was sent to Dr K. B. Raper in 1956 for his opinion and was examined by Dr Dorothy I. Fennell, who informed me that she concurred with my findings that the fungus is not *P. daleae*, but fits reasonably well with *P. lanosum*. *P. kojigenum* is quite unlike *P. lanosum*. Although definite fasciculation has not been demonstrated, the type of penicillus would tend to place it with the Fasciculata.

Penicillium madriti sp.nov. (Pl. 3, figs. 4, 5)

Coloniae in agaro Czapekii celeriter crescentes, viridi-coeruleae dein griseo-viridae, cum margine alba circa 2 mm. crassa, velutinae, guttulis numerosis, subflavis, reverso persicino, obscure zonato; coloniae in agaro 'malt' dictu celeriter crescentes, velutinae crassae, guttulis numerosis flavis, reverso badio; penicilli sine ramis, solum ex metulis et phialidibus compositi; conidiophora ex substrato, longa, laevia, 2–3 μ diam.; metulae 10–11 × 2·5–3 μ, apice subdilato; phialides 7–8 × 1·5–1·8 μ; conidia globosa, subasperula, 2–3 μ plerumque 2–2·5 μ diam.; catenae conidiorum in columnis digestae.

Hab. in solo hortense, Madriti; leg. G. Agosti. Typus L.S.H.T.M. BB389.

On Czapek agar grows fairly rapidly, pale blue-green, turning grey-green, with white edge about 2 mm. broad during the growing period, velvety but tending to become somewhat heaped in the centre; drops numerous, small, yellowish; reverse peach-coloured, indistinctly zoned; on malt agar grows very rapidly, greyish blue-green, thick velvety, with numerous yellow drops and reverse very reddish brown; penicilli with metulae each bearing a compact cluster of phialides, branching below the metulae not observed; conidiophores arising from the substrate, long, smooth, 2–3 μ diam.; metulae slightly swollen at the apex, 10–11 × 2·5–3 μ; phialides tapering rather gradually, but not acuminate as in the Biverticillata, 7–8 × 1·5–1·8 μ; conidia globose, finely roughened, 2–3 μ, mostly 2–2·5 μ diam., conidial chains forming a single fairly compact columnar mass, often bent or slightly twisted.

This species appears to form a transition between the *P. citrinum* and *P. chrysogenum* series. In the lack of any branching below the metulae it is nearest to *P. steckii* Zaleski, but the latter shows slower growth, thinner and more bluish colonies, and spore chains in several distinct columns, one to each metula. *P. madriti* was also grown in parallel cultures along with *P. cyaneofulvum* Dierckx, the member of the *P. chrysogenum* series which it most closely resembles. *P. cyaneofulvum* has thicker, almost floccose, colonies, penicilli which frequently show branching below the metulae, and much larger spores than *P. madriti.*

The type strain, maintained as Cat. No. BB389, was isolated in 1954, by G. Agosti in this Department, from garden soil from Madrid. A second strain, BB389A, was isolated from soil from a different garden in Madrid. On Czapek agar the latter forms slightly thicker colonies than 389, and rather more brownish colour in reverse; on malt agar the two are indistinguishable.

Aspergillus parvulus sp.nov. (Pl. 3, fig. 6)

Coloniae in agaro Czapekii vel aliis substratis rapide crescentes, cinnamomeae, velutinae, cum margine fimbriata, reverso flavo dein viridi-brunneo; capitula conidica parvissima, radiata; stipites breves ex hyphis repentibus, 15–30 μ longi, vel partes terminales hypharum repentium, sinuosi, 2–3 μ diam.; vesiculae pyriformes vel subglobosae, in toto fertiles, 4–9 μ diam.; metulae absentes; phialides paucae in capitulo, 5–6 × 2·5–3 μ; conidia globosa, subasperula, 3–3·3 μ diam.

Habitat in solo in silva permixta pini et Liquidambaris styracifluae, America boreali.

Grows well on all common culture media, spreading, light vinaceous cinnamon (Ridgway, Pl. XXIX), velvety, usually with a more or less fimbriate edge; reverse yellow, deepening in colour and finally becoming dull greenish brown; heads very small (hence the specific epithet), loosely radiate; stalks arising as short branches from surface hyphae, often with one septum, 15–30 μ long, originating from rather inconspicuous foot-cells, or as terminal portions of long trailing hyphae, and then without foot-cells, 2–3 μ diam.; vesicles varying from slight thickenings of the stalk to definitely pear-shaped or subglobose, fertile all over, 4–9 μ diam.; metulae entirely absent; phialides few in number, widely spaced, 5–6 × 2·5–3 μ; conidia globose, slightly rough, 3–3·3 μ diam.

A culture of this species was received, in June 1959, from NRRL, Peoria, as '*Scopulariopsis*? sp.'. It was isolated in 1942 by C. F. Andrus from the soil of a mixed pine and sweet gum forest. The type is maintained in the L.S.H.T.M. collection as BB405.

The position of this species in the Thom & Raper (1945) classification is difficult to assess. It does not in the least resemble any of the series in which metulae are lacking, the *Aspergillus clavatus*, *A. glaucus* and *A. fumigatus* series. The colour is very similar to that of many strains of *A. terreus*, but this species produces closely packed metulae and phialides, with the spores forming long solid columns.

It is becoming increasingly evident that the classification of the genus *Aspergillus* is in need of a thorough revision, in order to accommodate the various new species which have been described since 1945.

Aspergillus restrictus G. Smith mut. **eborinus** mut.nov.

Differt a *A. restricto* propter deficionem totam pigmenti atroviridis. In culto *A. restricti* inventus.

A culture of this organism, which arose as a spontaneous mutant in a culture of *A. restrictus*, was received from Mr E. Yuill in May 1945. It was soon lost, but a repeat transfer, received in October 1946, has remained quite typical. The type culture is in the L.S.H.T.M. collection as BB199.

Cultures on malt agar, or Czapek agar with 20 % sucrose, growing slowly, pure white, granular; spore-heads, as in the type strain of *A. restrictus*, forming long slender columns, often somewhat twisted; stalks colourless, widening slightly towards the vesicle, 4–6·5 μ diam.; vesicles pear-shaped, often merging gradually into the stalk, 10–15 μ diam.; phialides closely packed, borne only on the upper surface of the vesicle, with outer ones usually curved, 8–10 × 2·5–3 μ; conidia ovoid to pyriform when ripe, rough, 5–7·5 × 3–4 μ, frequently ripening slowly and then remaining for some time narrow and cylindrical, appearing as septate prolongations of the phialides.

Chaetomium fusisporum sp.nov. (Pl. 4, figs. 7–10)

Coloniae in agaro 'malt' dictu celeriter crescentes, dense floccosae, fulvae, cum plurimis nodosis myceliis sed peritheciis nullis; coloniae in agaro cum decocto tuberum celeriter crescentes, irregulariter fulvae et fuscae, cum peritheciis paucis; coloniae in agaro 'corn meal' dictu lente crescentes, tenuissimae, cum mycelio exiguo sed peritheciis numerosis; perithecia superficialia, subglobosa, atra, carbonacea, sine rostris, 130–200 μ diam., pilis terminalis longis, spiralibus, cum circa 18 flexionibus, asperis, 2–2·5 μ diam., et pilis lateralibus undatis, asperis, 100–200 μ longis, basi 2–2·5 μ diam. ad apicem contractis, praedita; asci evanescentes, clavati, octospori; sporidia fusoidea, brunnea, laevia, 14–15·5 × 6–7 μ.

Habitat in solo Australia occidentali; leg. J. H. Warcup.

On malt agar growing rapidly, matted floccose, tawny, with many mycelial knots suggestive of developing perithecia but no true perithecia; on potato agar similar, though with dark patches in the mycelium, producing perithecia spasmodically and scantily; on corn meal agar spreading slowly, with very little aerial mycelium, but soon producing fairly numerous perithecia; perithecia superficial, subglobose, black, carbonaceous, brittle, 130–200 μ diam.; terminal hairs long, unbranched, in fairly close spirals of up to about eighteen turns, very slender, only 2–2·5 μ diam., rough; lateral hairs and basal hairs slightly wavy, 100–200 μ long and 2–2·5 μ diam. at base, tapering towards the tip, rough; asci very evanescent, club-shaped, with the spores approximately biseriate; spores biconvex, fusoid, smooth, dark brown, 14–15·5 × 6–7 μ. The chief distinguishing features are the very slender, spiral perithecial hairs, and the large fusiform spores.

A culture of this species, along with a batch of cultures of other species of *Chaetomium*, was received in 1952 from Mr J. H. Warcup, Waite Institute, Adelaide. It was isolated from red-brown earth at the Institute and bore Warcup's number SA 64; it is maintained in the L.S.H.T.M. collection as the type, BB 382.

It is similar to *C. spirale* Zopf, but the spores of the latter are both shorter and wider, 9–11 × 7·5–9 μ, and are more rounded at the ends. The spores resemble those of *C. succineum* Ames, but in this the perithecial hairs are quite different. The spores are also similar to those of *C. fusiforme* Chivers, but here the hairs are not spirally coiled and are of greater diameter; also the perithecia are smaller.

ADDENDUM

Since this paper was submitted for publication a paper by Sergeeva (1960) has appeared, describing some new species of *Chaetomium*. One of these, *C. subspirilliferum*, appears from its description to be very similar to *C. fusiforme*. In both the terminal hairs are spirally coiled and the spores are similar in size. It is impossible to be sure whether the two are identical without seeing a culture of the Russian species, but the following table lists the apparent differences.

	C. fusiforme	*C. subspirilliferum*
Perithecia	Black, 130–200 μ, mostly 200 μ diam.	Olive to cinnamon 83–150 μ diam.
Terminal hairs	Uniformly in fairly close spirals	Lower third straight, upper part in loose spirals
Spores	Fusiform from all aspects	Concavo-convex; face view fusiform

The shape of the spores in *C. subspirilliferum* is given as convex on one face, concave on the other, broadly fusiform in face view. It is possible of course, that the concavity is produced by shrinkage in an unsuitable mounting medium, but, since no details of mounting methods are quoted, this again is problematical.

For the time being, at any rate, the two epithets should stand. It is to be hoped that a culture of *C. subspirilliferum* will become available for study in due course.

Eladia gen.nov.

Hyphae steriles repentes, crassae, hyalinae, septatae; conidiophora brevia, irregulariter ramosa, ramis ultimis phialides ferentibus; phialides dense compressae, breves, plus minusve ovoideae; conidia solitaria vel in catenis duorum triumve, asperula.

Species typicalis *Eladia saccula* (Dale) comb.nov. = *Penicillium sacculum* Dale apud Biourge in *Cellule*, **33**, fasc. 1, p. 323, 1923.

In 1914 Miss Dale described very briefly a fungus (D 6) which she had isolated from soil. It was tentatively regarded as a new species of *Penicillium* but was not named. It was sent to Biourge, who referred to it in his monograph of 1923, with a figure but without diagnosis, as *P. sacculum* Dale. In 1926 Miss Dale published a short note giving names for three species of *Penicillium* which had remained unidentified in 1914, one of the names being *P. sacculum*.

Thom (1930) stated that he had examined Miss Dale's D 6, and had reported to her that it was to be regarded as *Scopulariopsis repens* Bainier, but that he had subsequently lost the culture. Raper & Thom (1949) assign *P. sacculum* to *Scopulariopsis* on the basis of Thom's earlier findings, and without seeing and studying any fresh material.

In 1952 Mrs S. Marcus, working in this Department, isolated from a sample of mountain soil from Madrid a fungus which I did not regard as related to *Penicillium*, and which I submitted, under its catalogue number BB 298, to Mr E. W. Mason. He identified the mould as *P. sacculum* Dale, and subsequent examination of Miss Dale's and Biourge's figures confirmed this placing. It did not appear to me that its proper place is in *Scopulariopsis*, so, in view of the verdict in the *Manual*, it was sent to Dr Raper for his opinion. He replied that it is certainly not a *Penicillium* in the sense of the *Manual*, that it is nearer to *Scopulariopsis*, but is unusual in that the spores are borne singly or in very short chains.

Hughes (1953) has shown that *Scopulariopsis* is not related at all to *Penicillium*, since the two genera have entirely different methods of spore formation. In *Penicillium* the spores are phialospores, whilst in *Scopulariopsis* the spore-producing organ is an annellophore. It therefore became pertinent to re-examine the culture BB 298, in particular to determine whether the spore-producing cells are phialides or annellophores.

There are three characteristics which are useful in determining whether a spore-producing cell is an annellophore, *sensu* Hughes. First, the actual annellations may be detected, though this is usually far from easy. Secondly, an annellophore elongates slightly with every spore produced. Hence, on almost any slide of a typical *Scopulariopsis* it is possible to find conidium-producing cells of very varying length. This is, in fact, what had been noted, by many workers, in *Scopulariopsis* before the true method of spore-formation was suspected. Thirdly, annellospores are invariably truncate at the point of attachment.

A very careful examination of BB 298, using a good apochromatic objective and the highest useful magnification, has failed to detect annellations or any appreciable variation in length of the spore-producing cells. The latter are swollen, almost ovoid, not approximately cylindrical as in *Scopulariopsis*, and are remarkably uniform in length. It must be admitted that a large amount of variation in length, or the presence of any considerable number of annellations, would be impossible, since it is rare to find more than three conidia in a chain, and the amount of elongation per spore produced is small in *Scopulariopsis*. Nevertheless, the production of a chain of three spores should leave a detectable, even if very short, cylindrical portion on the end of the mother cell, and no such extension has been seen on any of hundreds of cells examined.

The spores of *Eladia saccula* are globose and spiny and, in mounted preparations, many of them bear what appears to be the initial of another conidium. This attachment certainly varies in size. When small it is more or less cup-shaped, exactly resembling approximately hemispherical buds seen on the tips of some of the phialides. When larger it is almost globose. Whatever the true nature of the attachment it is certainly not equivalent to the thickened basal ring in the spores of *Scopulariopsis brevicaulis*.

Since the diagnosis given by Miss Dale is quite inadequate, I append a more detailed description of *Eladia saccula*.

On malt agar growing moderately well, dull grey-green with white edge during the growing period, gradually becoming brownish green, velvety, slightly wrinkled; reverse greyish; colonies on potato agar growing somewhat more slowly, otherwise similar; colonies on Czapek agar thin and sparse, growing very slowly; on corn-meal agar growing slightly better than on Czapek agar, with colonies definitely arachnoid; on malt agar with 40 % sucrose growing slowly but colonies eventually typical, grey-green, smooth velvety; superficial hyphae funiculose, hyaline, coarse, septate more or less articulate, 3·5–6 μ diam.; sporing structures simple, usually consisting of a short conidiophore arising from one of the thick hyphae, unbranched or with one or two irregular branches each bearing a few closely packed phialides; phialides somewhat narrowed at the base, otherwise ovate, hyaline, 5·5–6 × 3–3·5 μ; conidia globose, brownish, densely spiny, usually with connective attached, 5–6μ diam.

Represented by two isolates: BB 298, as described above, and BB 298 A, received in June 1959 from NRRL, Peoria, as '*Acaulium* or *Scopulariopsis* sp.', obtained originally from A. L. Cohen, and isolated from soil of open field, Monterey, California.

Concerning Mucor vesiculosus *mihi, a correction*

Soon after the publication of the description of *M. vesiculosus* G. Smith (1957), my attention was drawn, by several correspondents, to the identity of this species with *Absidia butleri* Lendner. Dr C. W. Hesseltine, of Peoria, in correspondence, agreed with me that it is not correctly placed in *Absidia*, and suggested that it might be necessary to regard *Gongronella* Ribaldi as the correct place for it. In a synonymy sent to me by Dr Hesseltine there is, however, no mention of the actual transfer of *Absidia butleri* to *Gongronella* by Peyronel & Dal Vesco (1955), and later by Picci (1955).

The reasons for removing *A. butleri* from *Absidia* are:

(1) The subsporangial swelling is not the equivalent of the apophysis in the sporangia of *Absidia* spp. The latter is a region where the walls of the sporangium and the columella are fused together.

(2) The suspensors of the zygospores of *A. butleri* are completely devoid of ornamentation, the presence of long hairs on one or both suspensors being a diagnostic feature of *Absidia*. Dr Hesseltine kindly sent me mating strains of *A. butleri* for examination. My own strain (*M. vesiculosus*) proved to be minus.

The complete synonymy of this species, as far as it is known, is as follows:

GONGRONELLA BUTLERI (Lendner) Peyronel & Dal Vesco, in *Allionia*, **2**, 370, 1955.

Absidia butleri Lendner, in *Bull. Soc. bot. Genève*, Ser. II, **18**, 181, 1926.
A. subpoculata Paine, in *Mycologia*, **19**, 251, 1927.
Tieghemella subpoculata (Paine) Naumov, in *Clés des Mucorinées, Encycl. mycol.* **9**, 79, 1939.
T. butleri (Lendner) Naumov, in *Clés des Mucorinées*, 79, 1939.
Gongronella urceolifera Ribaldi, in *Riv. Biol. gen.*, N.S. **44**, 164, 1952.
Mucor vesiculosus G. Smith, in *Trans. Brit. mycol. Soc.*, **40**, 481, 1957.

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Thom, C. & Raper, K. B. (1945). *A manual of the Aspergilli.* London: Baillière, Tindall and Cox.

EXPLANATION OF PLATES 3 AND 4

Plate 3

Fig. 1. *Penicillium kewense.* Penicillus, × 1000.
Fig. 2. *P. kewense.* Asci and ascospores, × 1000.
Fig. 3. *P. kojigenum.* Penicillus, × 1000.
Fig. 4. *P. madriti.* Penicillus, × 1000.
Fig. 5. *P. madriti.* 2 penicilli, × 1000.
Fig. 6. *Aspergillus parvulus.* Typical head, × 1000.

Plate 4

Fig. 7. *Chaetomium fusisporum.* Perithecium, × 100.
Fig. 8. *C. fusisporum.* Perithecial hairs, × 500.
Fig. 9. *C. fusisporum.* Young asci, × 1000.
Fig. 10. *C. fusisporum.* Spores, × 1000.

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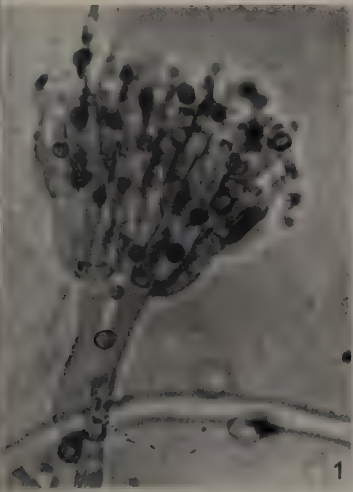
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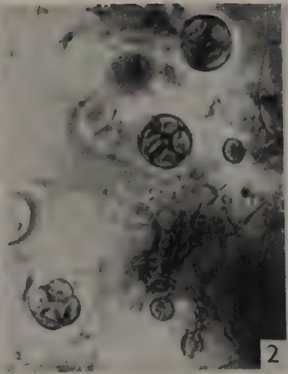
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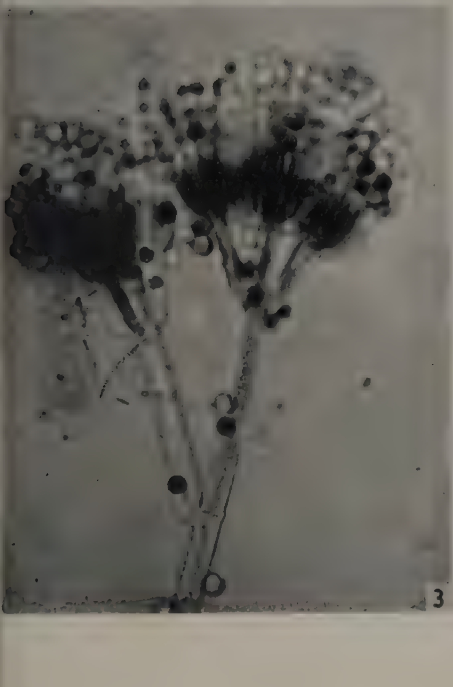
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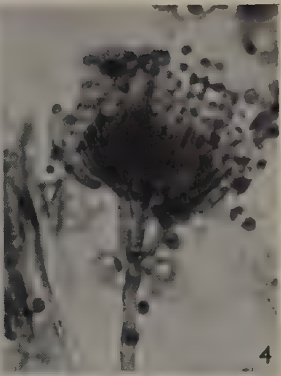
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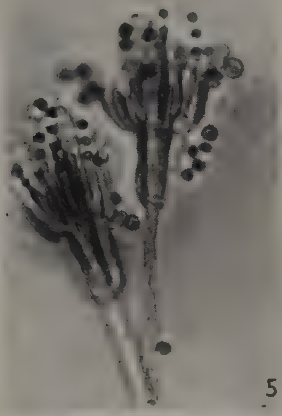
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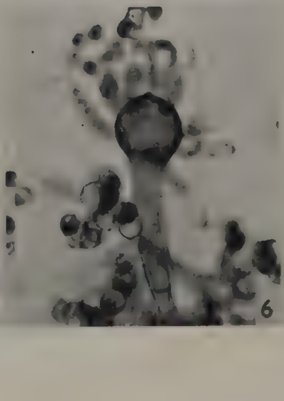
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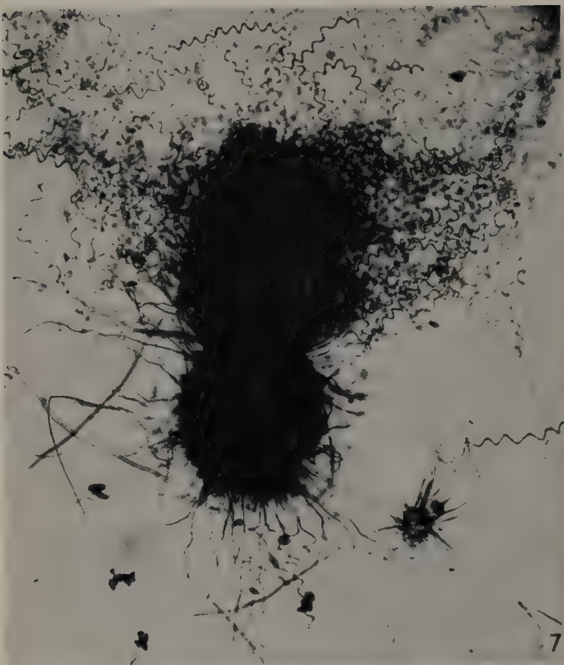

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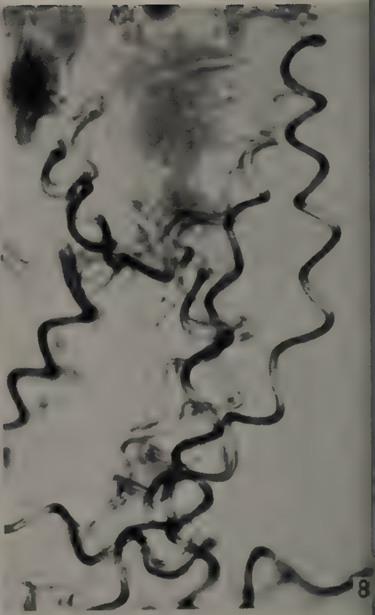

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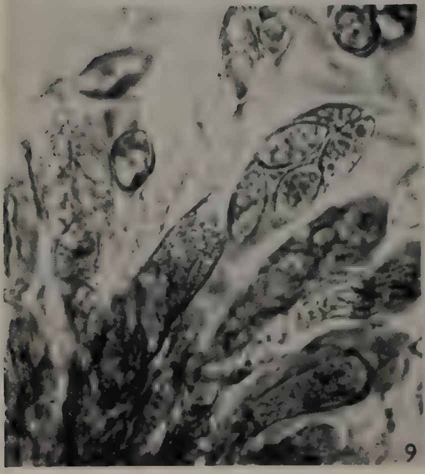

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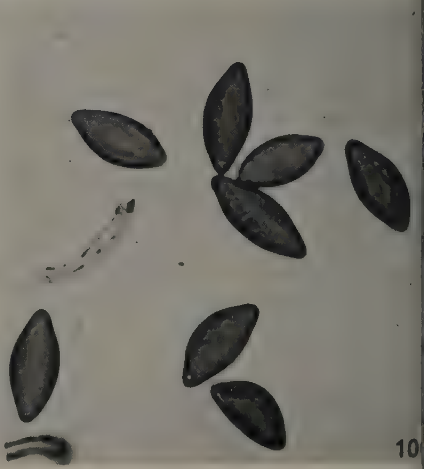

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Trans. Brit. mycol. Soc. **44** (1), 51–54 (1961).

NOTES ON FUNGI FROM NORTH-EAST INDIA

V. A NEW GENUS OF THE HELICOSPOROUS HYPHOMYCETES

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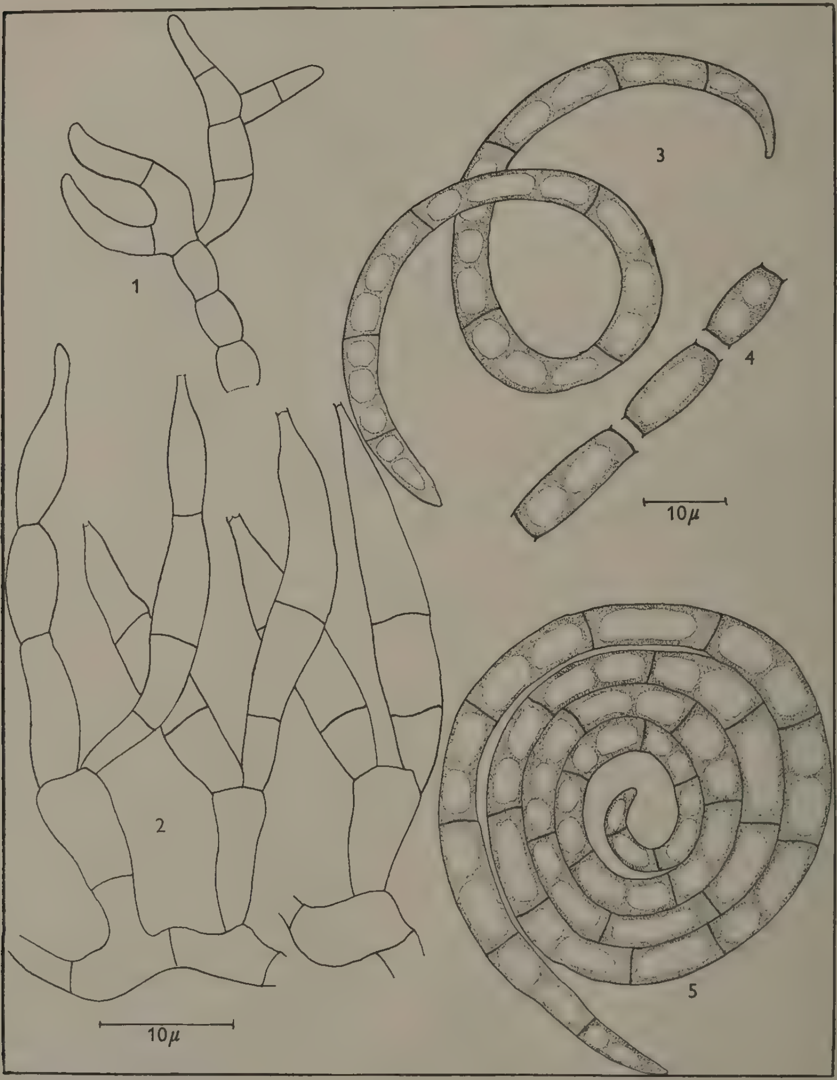
(With 1 Text-figure)

A very interesting tuberculariaceous fungus has been repeatedly collected during the rainy months (May–Sept.) in the last 3 years or so on decaying wood. It was collected once on decaying frames of an uprooted tea bush on the Cinnamara Tea Estate, Assam, in 1957, but not on tea since then. Although the fungus was usually collected in a pure stand, sometimes, nevertheless, it was found in association with an undetermined species of *Helicosporium*.

The sporodochia of the fungus to begin with are pin-point-like structures, white and glistening. With the production of the conidia they turn pale pinkish or buff and become pulvinate. The fruit bodies are entirely superficial and are dislodged by a mere flick of the needle. The sporodochia, 200–600 μ, are hemispherical to subspherical with a deeply umbilicate centre. The conidia are produced in a centrifugal succession from the centre to the periphery on hyaline to subhyaline fasciculate conidiophores measuring up to 30 × 2–5 μ; they are very rarely forked, up to 3-septate, bearing conidia singly and terminally. The conidial filament is 3–7 (mostly 6) times coiled. The filaments of the helicospore are coiled in two dimensions and are invariably curved towards the centre of the sporulating sporodochium so that it presents a deeply umbilicate centre. The conidia are up to 70 μ diam., mostly 50 μ, the filaments being narrower at the extremities. Mature conidia were observed to fragment in some instances. Germination of the spores was not obtained in distilled water. All attempts to culture the fungus on common laboratory media and sterilized pieces of wood on which the fungus was first collected were unsuccessful.

A search through published literature on the helicosporous hyphomycetes revealed no genus in which the above fungus could be placed conveniently. Conidia are coiled in three dimensions in *Troposporium* Harkness and *Hobsonia* Berkeley (Linder, 1929). In *Delortia* Patouillard sporodochia are gelatinous and conidiophores are swollen at the apex below the conidia to form an apophysis-like structure; the conidia are coiled in two dimensions as in the fungus under report but the filaments are 2–4 septate and only once coiled (Linder, 1929). *Troposporella* Karsten has conidia which are coiled in two dimensions; the sporodochia are subspherical or pulvinate and the surface farinaceous and olivaceo-fuligeneous, not buff as in the fungus occurring locally. In addition, the conidiophores are moniliform, articulate and subfuligineous and the conidial filaments $1\frac{1}{2}$–2 times coiled and 12–16μ diam. (Linder, 1929). *Everhartia* Saccardo & Ellis has sporodochia which are verrucose, fuscous, superficial, densely conglobate and very often stipitate to substipitate.



Figs. 1–5. *Vanbeverwijkia spirospora* Agnihothrudu. 1. Substrate mycelium. 2. Conidiophores. 3. Conidium. 4. Conidial fragments. 5. A helicospore. (All drawn from Mycol. Herb. Tocklai Exp. Sta., no. 168.)

Drepanoconis Schroeter & Hennings has only two species. In *D. larvaeformis* Spegazzini the conidia are only $\frac{1}{2}$–1 coiled and the conidiophores are well developed. In *D. anguisporus* (Pat. & Lagerh.) Linder the conidia are almost sessile. The spore filaments of both these species are up to 18 μ thick (Linder, 1929). As the fungus described above does not fit into any of these genera a new genus is proposed to accommodate it.

Vanbeverwijkia gen.nov.

Fungus Imperfectus, Tuberculariaceae, Helicosporeae; sporodochia discrete, hemispherical to subglobose, superficial; conidia closely coiled in two dimensions, hyaline, multiseptate, borne on densely aggregated conidiophores.

The generic name is after Miss Agathe L. van Beverwijk, Centraalbureau voor Schimmelcultures, Baarn, whose contributions to our knowledge of helicosporous hyphomycetes are well known. Type species

Vanbeverwijkia spirospora sp.nov.

Sporodochia minute, whitish at first, becoming buff, gregarious to scattered, superficial, tuberculiform, 200–600 μ diam., hemispherical to subglobose, deeply umbilicate in centre. Conidiophores short, hyaline to subhyaline, fasciculate, very rarely forked, septate, arising from a densely compacted substrate mycelium 16–30 × 2–5 μ; conidia hyaline, closely coiled 3–7 times in two dimensions, multiseptate, 40–70 μ diam., the filaments about 6 μ in the middle and 3–4 μ at the extremities, guttulate. The conidia are produced singly and terminally on the conidiophores.

Type on decaying wood, Jorhat, Assam, collected by Mr H. K. Phukan, 18 August 1958, deposited in the Mycological Herbarium, Tocklai Experimental Station. No. 168; isotype in Herbarium Cryptogamiae Indiae Orientalis, New Delhi.

Vanbeverwijkia gen.nov.

Pertinet ad Fungos Imperfectos, ad Tuberculariaceas, Helicosporas. Sporodochia discreta, hemisphaerica ad subglobosa superficialia; conidia arcte spiraliter disposita dimensione duplici, hyalina, multiseptata, insidentia conidiophoris arcte aggregatis. Species typica

Vanbeverwijkia spirospora sp.nov.

Sporodochia minuta, primo albida, evadentia bubalina, gregaria vel dispersa, superficialia, tuberculiformia, magnit. 200–600 μ diam., hemisphaerica vel subglobosa, alte umbilicata in medio. Conidiophora brevia, hyalina vel subhyalina, fasciculata, rarissime furcata, septata, emergentes e mycelio dense compacto, magnit. 16–30 × 2–5 μ; conidia hyalina, arcte spiraliter ter ad septies curvata, duplicis dimensionis, multiseptata, magnit. 40–70 μ diam., filamentis ca. 6 μ in medio, 3–4 μ ad apices, guttulata. Conidia producta singulariter atque terminaliter conidiophoris insidentia.

Typus lectus in ligno tabescente, ad Jorhat in Assamia a H. K. Phukan 18 augusti 1958 et positus in herbario Stationis Experimentalis Tocklai sub numero 168; isotypus in Herbario Cryptogamiae Indiae Orientalis, New Delhi.

I am grateful to the Director, Tocklai Experimental Station, for permission to publish this paper; to Mr K. C. Sarmah, the Mycologist, for his encouragement; to Miss Agathe L. van Beverwijk, Director, Centraalbureau voor Schimmelcultures, for critically reviewing the manuscript; and to Prof. Dr Rev. Fr. H. Santapau for rendering the diagnosis into Latin.

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Trans. Brit. mycol. Soc. **44** (1), 55–71 (1961).

THREE SPECIES OF *DIDYMOSPHAERIA* ON MARINE ALGAE: *D. DANICA* (BERLESE) COMB.NOV., *D. PELVETIANA* SUTH. AND *D. FUCICOLA* SUTH.

By IRENE M. WILSON and J. MARY KNOYLE*
University College of Wales, Aberystwyth

(With 39 Text-figures)

A historical review is given of *Leptosphaeria marina* Rostrup, 1889, on *Chondrus crispus*. As this name was already in use in 1889, the first valid name was *L. danica* Berlese, 1892. In 1913 Lind pointed out that the spores were bi-celled and transferred it as *Didymosphaeria marina* (Rostr.) Lind. It is suggested that its correct name is *D. danica* (Berlese) comb.nov.

A re-investigation of *D. danica* supports the assignation to *Didymosphaeria* since the spores are always two-celled and are capable of developing pigment, though usually hyaline. A brief comparison with *D. pelvetiana* Suth. on *Pelvetia canaliculata* and *D. fucicola* on *Fucus vesiculosus* shows that these three fungi are closely related. They are compared with members of some other genera and their systematic position in the bitunicate Ascomycetes with the *Pleospora*-type of development is indicated.

Cercospora salina Suth. has been repeatedly isolated from the blackened clypeus region of *D. danica* and is regarded as a secondary saprophyte.

In 1889 Rostrup described a new pyrenomycetous fungus on thalli of the red seaweed *Chondrus crispus*, which had been washed up on the shore at Klitmøller, Denmark. The black spots caused by the fungus contained septate, branched hyphae and several perithecia with asci surrounded by thread-like paraphyses. The ascospores were hyaline or very pale yellow and had a distinct cross-wall, but in some there was thought to be an indication of three cross-walls. Rostrup therefore considered that his specimen was an immature *Leptosphaeria* and named it *L. marina*.

Saccardo (1891) listed Rostrup's *L. marina* on *C. crispus* and gave measurements of the (immature?) ascus as 50 × 10 μ and of the ascospores as 16 × 4 μ. In the same publication he referred to Ellis & Everhart's *L. marina* (1885) on *Spartina*, thus showing that Rostrup's binomial had already been used for another species.

Berlese (1892) therefore proposed *L. danica* for *L. marina* Rostrup 1889 (non. Ell. & Ev. 1885). This is its first valid name and Rostrup's specimen is the type.

Jones (1898), unaware of the European literature, described the same fungus as a new species, *Sphaerella chondri*. His material was collected during November and December at Nahant, Mass. His measurements for

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* Now at Glaxo Laboratories, Sefton Park, Slough, Bucks.

the asci were 75–80 × 11–13 μ and for the ascospores 33–34 × 5–6 μ. He described the spores as hyaline and uniseptate with the upper cell more acute. Paraphyses were not mentioned.

Rosenvinge (1906) found the fungus in several places on the northern shores of Denmark, always confined to *C. crispus* and on living plants submerged under the sea as well as on thalli washed up on the shore. He pointed out that the fungus infected only the cystocarpic and tetrasporic pustules. He thought that infection occurred after a considerable quantity of carpospores or tetraspores had been discharged and that the hyphae attacked the remaining spores and penetrated into them. He described for the first time the imperfect, '*Phoma*-like' pycnidial state in specimens collected from June to September. The pycnidia were 156–175 × 100 μ and contained thin conidiophores which abstricted colourless, rod-shaped conidia, 4 × 1 μ. The perithecia occurred on specimens collected from July to September and were sometimes found in the same blackened pustules as the pycnidia. The perithecia were 125–215 × 100–300 μ and the ascospores were uniseptate, hyaline and 29–31 × 5–6 μ. There were thin, somewhat branched paraphyses. The perithecia were sunk in the rind of the *Chondrus* thallus which was penetrated by dark hyphae and therefore appeared black, and the black hyphae were also found in the apex of the perithecium; the rest of the perithecial wall was colourless.

Like Rostrup, Rosenvinge thought that his specimens were immature and retained the generic name *Leptosphaeria*, but, as Rostrup's name *L. marina* had already been used by Ellis & Everhart, he proposed the new name *L. chondri* (Rostr.) Rosenv. He did not refer to the earlier names, *L. danica* or *Sphaerella chondri*.

Cotton (1908) referred to the work of Rostrup, Jones and Rosenvinge and gave a re-description under the name *L. chondri*. The name *Leptosphaeria* was retained since Cotton, like Rostrup and Rosenvinge, considered the spores might be immature: *Sphaerella* was ruled out because of the presence of paraphyses. Cotton's material was obtained from fronds of *C. crispus* washed up on the shore at Swanage, Dorset, in February, and he noted the wide distribution of the fungus on both sides of the Atlantic. He considered that infection occurred near the cystocarps and tetrasporic pustules at an early stage and that entry through openings caused by discharge of carpospores or tetraspores was improbable. Both pycnidia and perithecia were present on the pustules. The asci were difficult to observe owing to the early deliquescence of the walls and the ascospores were said to be hyaline, unequally two-celled, and 25–40 × 5–7 μ (immature).

Lind (1913) reviewed all the previous literature, pointing out that apparently mature, *brown* ascospores were 1-septate and that there was no real evidence that they ever had more than one septum. The fungus was therefore not a *Leptosphaeria*. After rejecting the possibility that it might be a *Dothidella* because a stroma was not formed before the perithecia and because of the presence of paraphyses, he transferred it to *Didymosphaeria* as *D. marina* (Rostrup) Lind.

Knoyle (1951) re-examined the fungus on *Chondrus*, confirmed that the spores were 1-septate and showed that they germinated in this condition. The present paper is a corrected and extended account of this work together with drawings of the fungus (since none exists in the literature). Some observations on two allied species on *Pelvetia* and *Fucus* are included for comparison.

Didymosphaeria danica (Berlese) comb.nov. (syn. *Leptosphaeria danica* Berl. *Icones Fungorum*, **1** (2), p. 87. 1892).

Infected material of *Chondrus crispus* (L.) Stakh. from England, Scotland, Wales, Brittany and Massachusetts, U.S.A., was examined. At Aberystwyth *C. crispus* is common on the College Rocks. There the littoral form with broad, membranous thalli, found in rock pools, is rarely attacked by the fungus. The more cartilaginous sublittoral form found on the steep seaward-facing rocks, fully exposed to the open sea, is completely clear of infection, but in the sheltered lagoon-like area north of the pier, exposed to some contamination from buildings on the pier, where *Chondrus* and other small algae form an undergrowth below *Laminaria digitata* and are accessible only at low spring tides, a high proportion of the plants is infected. All the illustrations here are from material collected from this last area or from drift weed washed up on the shore in its neighbourhood.

Fresh material was collected every month and all stages were examined in the fresh condition. Material was also fixed in Flemming's fluid, embedded in paraffin wax, microtomed and stained with Heidenhain's haematoxylin counterstained with erythrosin, or with cotton blue and erythrosin. Stock material for hand sections was fixed in 4 % formalin in sea water. The freezing microtome was useful for sections of fresh material.

Relationship of host and fungus

The littoral form of *C. crispus* has a winter climax of tetraspore production and a summer climax of carpospore production (Marshall, Newton & Orr, 1949; Devonald, 1950). The peaks for the sublittoral form (Knoyle, 1951) appear to be somewhat later (Figs. 1, 2). There is, however, considerable overlapping of the fruiting periods and both tetrasporic and cystocarpic plants can be found at all times of the year. The fungus is confined to the fruiting parts of the alga and can be found all the year round too, both on living plants and on plants washed up on the shore. Attack is not confined to pustules from which carpospores or tetraspores have already been shed, but old plants are more frequently and more heavily infected.

The fungus starts its growth as a parasite on living plants; though it is frequently found on detached plants, there is no evidence that it can spread from one detached plant to another. It appears to have specialized nutritional requirements since it is confined to the fruiting parts of this single algal host.

There is only rarely any marked distortion of the host tissues. The presence of the fungus can be recognized by the small black spots on the cystocarps and tetrasporic pustules (Figs. 3–7), the smaller spots representing the position of pycnidia and the larger ones the perithecia.

The pycnidia are evidently formed first (Rosenvinge, 1906; Jones, 1898; Cotton, 1908). Knoyle (1951) found, in contrast to the reports in the literature, that pycnidia were present on living, submerged *Chondrus* in greater quantity from July to September and perithecia from October to March. In an attempt to elucidate these conflicting results an analysis of drift material was made throughout 1957. Both pycnidia and perithecia with ascospores were found in every month of the year, but perithecia were more likely to be found on the older cystocarpic and tetrasporic plants. The fruiting of the fungus does not therefore appear to be directly governed by the seasons but rather by the fruiting periods of the algal host, but since these show seasonal high levels of tetraspore and carpospore production (although these fruiting periods are very prolonged and overlap) there is an indirect seasonal effect on the fungus.

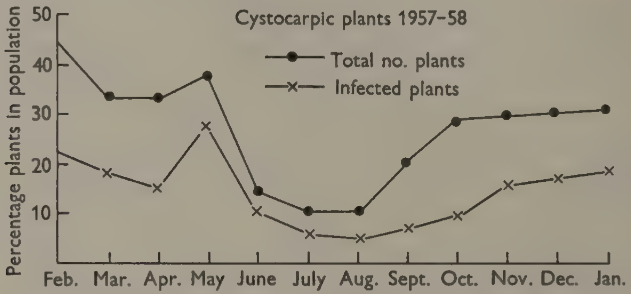


Fig. 1. Cystocarpic plants of *Chondrus crispus* in sublittoral population. Cystocarps begin to appear on new growth in March, the rate increases in Aug. and Sept., remains high until the end of Jan. and by June the old cystocarps have disappeared. A subsidiary peak in May is due to the formation of new cystocarps, some already infected, while the old ones, many heavily infected, still exist.

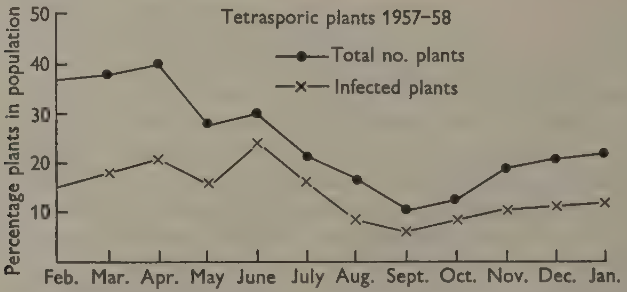
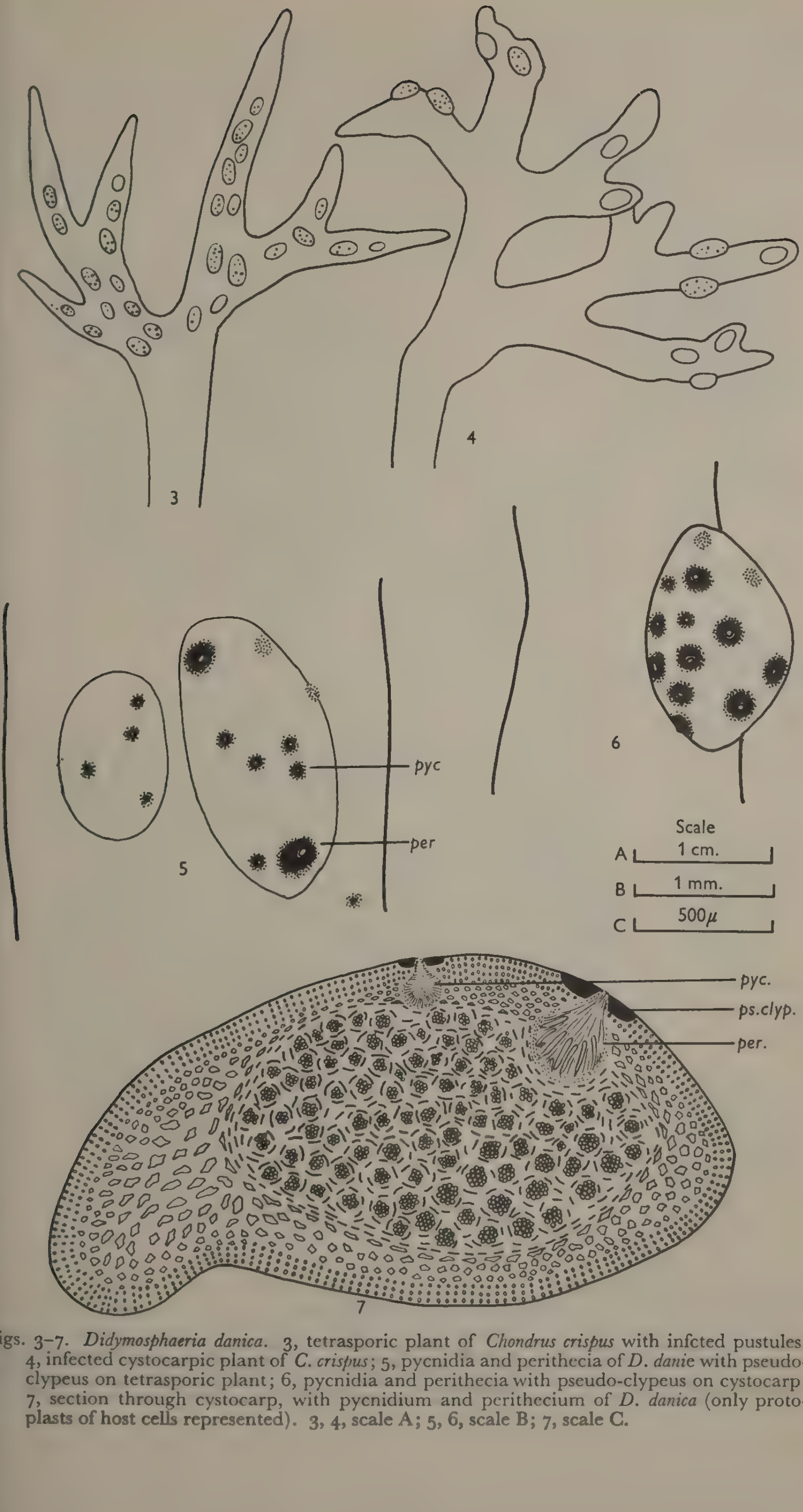


Fig. 2. Tetrasporic plants of *Chondrus crispus* in sublittoral population. Tetrasporangia begin to form on new thallus tips towards end of May, rate of formation increases steadily and numbers remain high during winter months, declining after April. The subsidiary peak in the curve in June is due to formation of new tetrasporic pustules on new growth while old plants are still declining.

The mycelium in relation to the host tissues

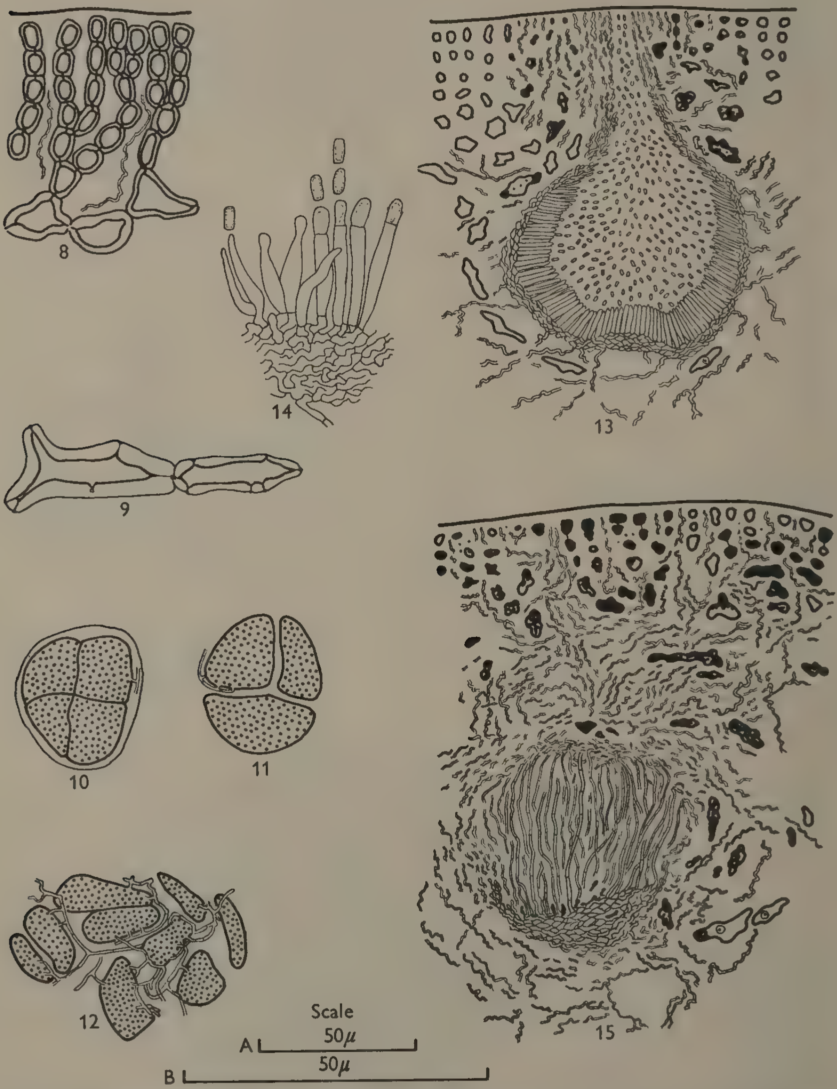
The thallus of *C. crispus* is a multiaxial structure (Figs. 7–9). The central medulla consists of filaments of elongated cells connected to each other at their narrowed ends by primary pit connexions and to the other filaments by secondary pit connexions (Figs. 8, 9). Near the surface these axial



Figs. 3–7. *Didymosphaeria danica.* 3, tetrasporic plant of *Chondrus crispus* with infcted pustules; 4, infected cystocarpic plant of *C. crispus*; 5, pycnidia and perithecia of *D. danie* with pseudo-clypeus on tetrasporic plant; 6, pycnidia and perithecia with pseudo-clypeus on cystocarp; 7, section through cystocarp, with pycnidium and perithecium of *D. danica* (only protoplasts of host cells represented). 3, 4, scale A; 5, 6, scale B; 7, scale C.

threads bear numerous perpendicular small-celled laterals which form a compact cortex, separated from the central conducting region by cells of intermediate size and all joined by pit connexions. There is a firm 'cuticle' at the surface (Fritsch, 1945).

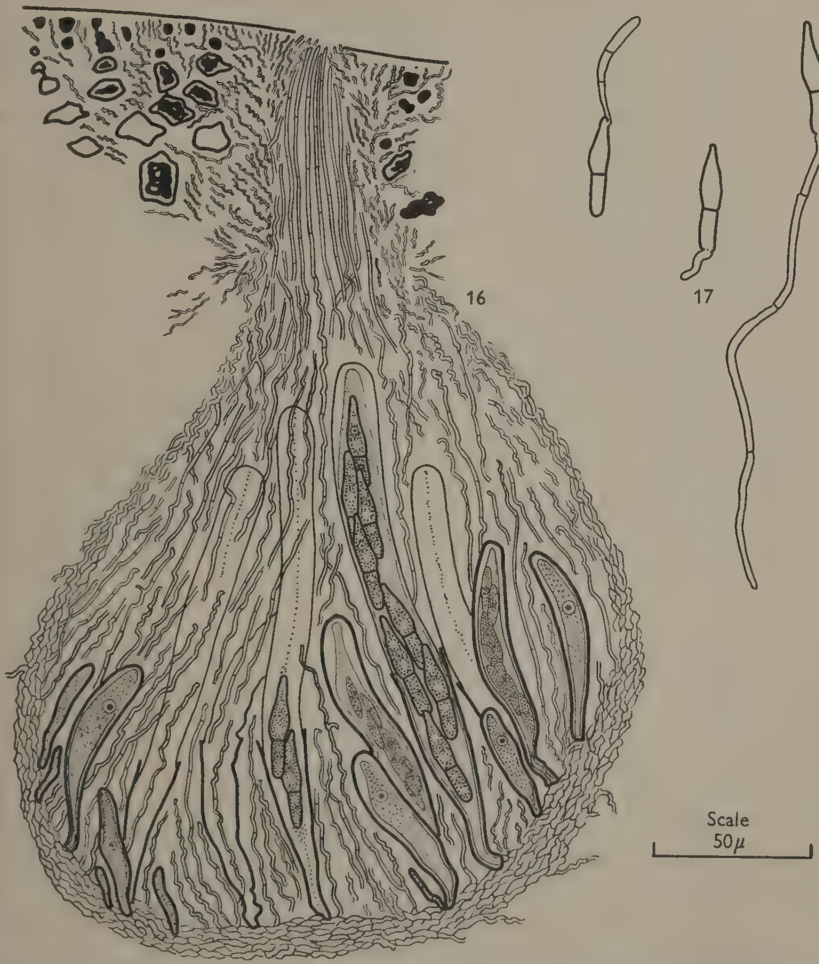
The wall of each host cell consists of an outer layer composed of pectic



Figs. 8–15. *Didymosphaeria danica*. 8, intercellular hyphae in cortical rind of living *Chondrus*; 9, Medullary cells of living *Chondrus* showing Floridean pits (in 8 and 9 wall layers protoplast, and primary and secondary pit connexions shown); 10, hypha in wall of tetrasporangium; 11, hypha on tetraspores; 12, hyphae and carpospores; 13, section through pycnidium (only protoplasts of host shown); 14, conidiophores and conidia; 15, section through young ascocarp (only protoplasts of host shown); 8–13, 15, scale A; 14, scale B.

substances and an inner consisting largely of cellulose (Figs. 8, 9). The wall swells during the preparation of permanent slides and on heating in water forms, with the intercellular matrix, a jelly-like substance. Carageen is derived from the intercellular matrix (Kylin, 1956).

The mycelium is localized, being confined to the cystocarpic and tetrasporic pustules. The pycnidia and perithecia are formed over these fruiting regions or occasionally on the thallus close to them. Some pustules have only pycnidia or only perithecia, but the majority have both (Figs. 5, 6).



Figs. 16–17. *Didymosphaeria danica.* 16, section through ascocarp with asci at various stages of development (only protoplasts of host shown); 17, ascospores germinating in sea water.

The mycelium is about 1 μ diam., colourless to very pale yellow in mass, septate and branched. It ramifies in the intercellular matrix (Figs. 8, 13, 15, 16). As each fungal fruit body develops, the mycelium extends up to the surface of the host and becomes particularly abundant round the ostiole where it causes blackening of the protoplast, yellowing of the walls and a disorderly arrangement and partial replacement of the cortical cells. These blackened host cells and the mycelium together form the black spot visible externally (Figs. 3–7, 13, 15, 16). This may be called a pseudo-clypeus, following the usage of Sutherland (1915), or, according to more modern taxonomic practice in which no distinction is made between a clypeus consisting exclusively of fungal hyphae and a pseudo-clypeus where a high proportion of host tissue remains, simply a clypeus.

The ostiolar region may be invaded by coarse, brown hyphae which were considered by Rosenvinge (1906) to belong to *Leptosphaeria chondri* (as he called the fungus). Since these dark hyphae are not invariably present, though they are very frequent on drift material during the summer months, and since *Cercospora salina* Suth. has been isolated regularly and repeatedly from the region infected by these dark hyphae, they are considered to represent a secondary infection by this common saprophyte on other marine fungi.

The mycelium of *Didymosphaeria danica* also extends downwards into the fruit body of the alga and ramifies between and around the carposporangia or tetrasporangia. It appears to penetrate the wall and to send branches into the protoplast itself, so that when the carpospores or tetraspores are released as naked cells from their enveloping wall they may still bear the mycelium of the fungus round them (Figs. 10–12). Such spores do not germinate to form infected sporelings, as may occur in the *Ascophyllum nodosum*/*Mycosphaerella ascophylli* or the *Pelvetia canaliculata*/*M. pelvetiae* complex (Cotton, 1908; Sutherland, 1915); they are damaged by the parasite and frequently appear blackened.

The pycnidium

The pycnidium is flask-shaped (Fig. 13) with a neck of variable length and measures 100–192 × 63–113 μ (average 138 × 85 μ). Interwoven hyphae form the pale, small-celled wall round the base and sides of the pycnidium while the ostiolar region is less definitely delimited by vertically running hyphae and is surrounded by the pseudo-clypeus of blackened and partially disorganized host cells and numerous intercellular hyphae. The base and sides of the pycnidium are lined by long, narrow or slightly flask-shaped conidiophores, approximately 16–18 μ long (Fig. 13), which are unbranched, except very occasionally at the base (Fig. 14). The conidia are hyaline, oblong and approx. 4 × 1 μ. They are formed in long, mucilaginous, basipetal strings from the apex of the conidiophores.

The pseudothecium

In the literature the ascocarp is referred to as a perithecium. The observations of the development and structure recorded below suggest, however, that it is not a true perithecium in the modern sense but rather an ascostroma or pseudothecium (Nannfeldt, 1932; Miller, 1949; Luttrell, 1951, 1955).

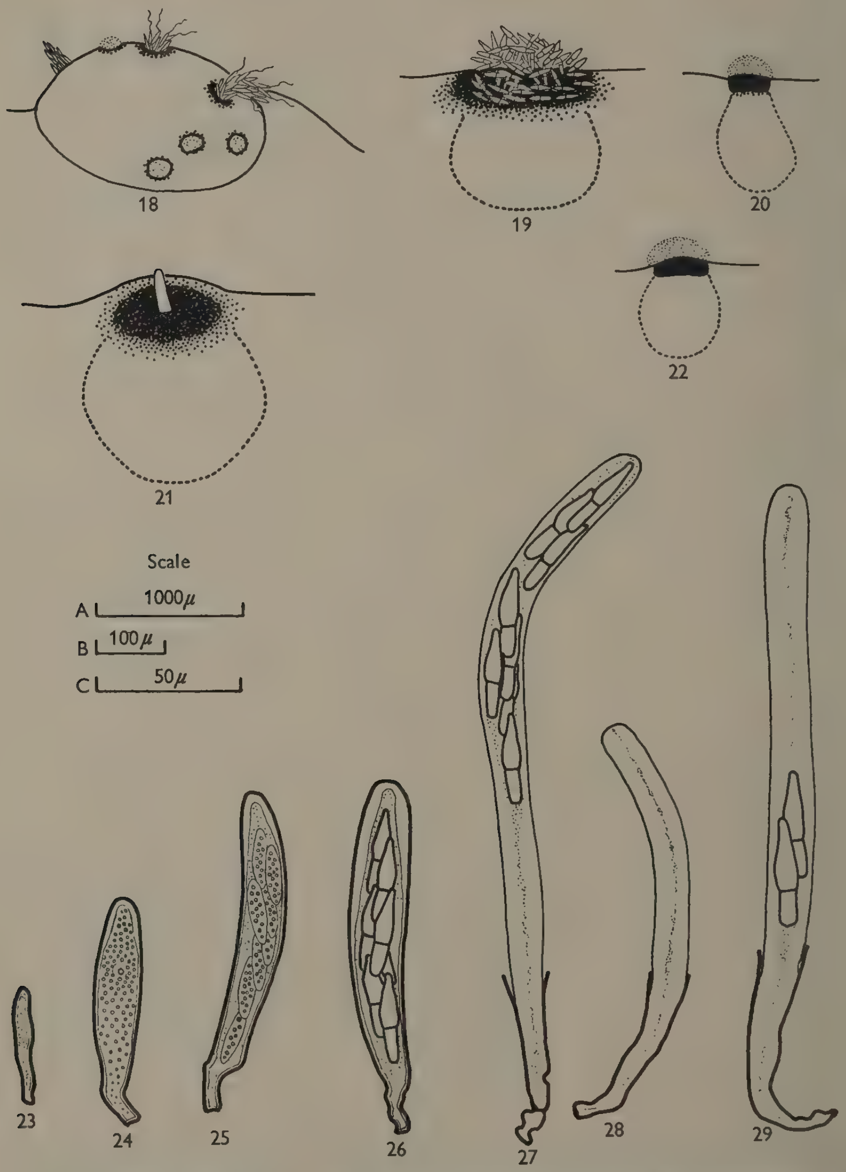
The youngest stage observed was a small, fairly compact mass of interwoven hyphae between the cortical rind and the fruiting portion of the alga, and already differentiated into an outer 'wall' layer and inner, more deeply staining and more vertically arranged hyphae. As this small stromal knot enlarges and digests the host cells, it becomes globose (Fig. 15), loosely bounded on the outside by numerous fine, branching hyphae which are more densely woven at the top and bottom than at the sides. Within the centrum there are vertical hyphae, the pseudoparaphyses, which, in the stage shown in Fig. 15, are already attached both above and below. Growing up from the base are the deeply staining ascogenous hyphae which will later give rise to asci. From the young fruit body numerous fine, branching hyphae radiate out through the intercellular spaces of the host, reaching the surface of the thallus above and penetrating into the fruiting portion below and causing discoloration and partial disorganization of the host cells in both regions.

As the globose fruit body enlarges the upper part pushes upwards as a dome-shaped projection to form the neck. As it does so, many of the pseudoparaphyses are stretched and broken. Meanwhile the asci have developed from the binucleate cells of the ascogenous hyphae. It is doubtful whether croziers are formed, but a succession of asci develops, one below the other on the ascogenous hyphae.

The mature fruit body is flask-shaped (Fig. 16) and measures 180–267 × 156–277 μ. The wall round the venter is a firm, pale, small-celled tissue formed of interwoven hyphae, but it becomes less distinct above and is very indefinite in the neck and ostiolar region where it is surrounded by the pseudo-clypeus of blackened and partially disorganized host cells and numerous fine, branching, intercellular hyphae. The numerous pseudoparaphyses which broke during the expansion of the centrum contract to form fine, wavy strands standing up among, and taller than the asci or hanging down from the top and sides of the inside of the fruit body. They are fine, hyaline, probably septate hyphae, sometimes branched and occasionally anastomosing, and, in fresh material, are seen to contain a row of minute oil globules or granules of some sort.

The asci are numerous and arise from the base and lower part of the sides of the fruit body. They measure 83–94 × 12–14 μ and contain eight irregularly biseriate ascospores which fill most of the lumen of the ascus with only a very short narrow pedicel below. The asci are bitunicate and dehisce by the usual 'Jack-in-the-box' mechanism. There is a thin, firm ectoascus which breaks at dehiscence and an endoascus which becomes thickened near the apex after the delimitation of the spores and later stretches up to the ostiole during dehiscence (Figs. 16, 21, 23–29). A thimble-shaped cap as in *Leptosphaeria discors* (Johnson, 1956) is not usually formed.

The ascospores are 33–40 × 5–7 μ (exceptionally 44 × 8 μ, perhaps just before germination). In frequent examinations of the fungus over the past 15 years from different countries, from different localities in Britain, from littoral and sublittoral *Chondrus*, attached and in the drift, spores with more than one septum have never been seen; they are normally discharged and germinate in the bicellular condition. The lower cell is more or less cylindrical and the upper cell longer and more pointed. The extreme apex of the upper cell is rounded and may become rather pale and swollen as if it were mucilaginous. The spores are usually completely hyaline, but may



Figs. 18–29. *Didymosphaeria danica*. 18, part of cystocarpic thallus of *Chondrus* submerged in sea water; conidia oozing but not germinating; ascospores, still one-celled, oozing and germinating; 19, 20, in sea water, ascospores and conidia at ostioles; 21, 22, in damp atmosphere, ascus projecting through ostiole and conidia at ostiole; 23–29, stages in development of ascus. 18, scale A; 19–22, scale B; 23–29, scale C.

become slightly yellow; no brown spores, as recorded by Lind (1913), have been observed. Coloration of the spores, if it occurs at all, takes place outside the fruit body, but in the sea they germinate at once while still hyaline.

Discharge, dispersal and germination of the reproductive units

Whether the host thallus is submerged under sea water or kept in a damp atmosphere (Figs. 20, 22), the conidia ooze in a mucilaginous mass through the ostiole of the pycnidium and remain there until swept away by the sea. They have not been seen to germinate in sea water, even when kept under observation for 5 days after discharge, and their function remains unknown.

When thalli bearing ascocarps with asci are kept in a damp atmosphere or under sea water, ascospores tend to accumulate in a heap over the ostiole (Figs. 18, 19). If the asci in such ascocarps are examined, they are found to have dehisced in the normal manner (Figs. 23–29), and in a damp atmosphere stretched asci may be seen protruding through the ostiole (Fig. 21). The bitunicate ascus therefore functions quite normally both in damp air and submerged, but there seems to be little force behind the discharge. This is unimportant in a marine fungus since, as long as there is a mechanism to bring the reproductive units to the surface, the sea can wash them away. If sections of ascocarps are cut and mounted so that the asci are in direct contact with water, the inner wall of the ascus may swell without stretching and the spores, including immature, one-celled spores, are then readily squeezed out of the asci in packets of eight. Such spores may ooze in a rod from the ostiole.

The ascospores germinate readily within about 48 hr. in a shallow layer of sea water or on a damp surface, adequate aeration being necessary. The spores are normally bi-celled when they germinate, but apparently immature, one-celled spores can germinate too. The first stage in germination is the disappearance of the larger granules and the appearance of fine, homogeneous cytoplasm. A germ tube is put out subterminally from either the upper or the lower cell of the spore, but not from both. It grows long and straight, without branching, in sea water (Fig. 17).

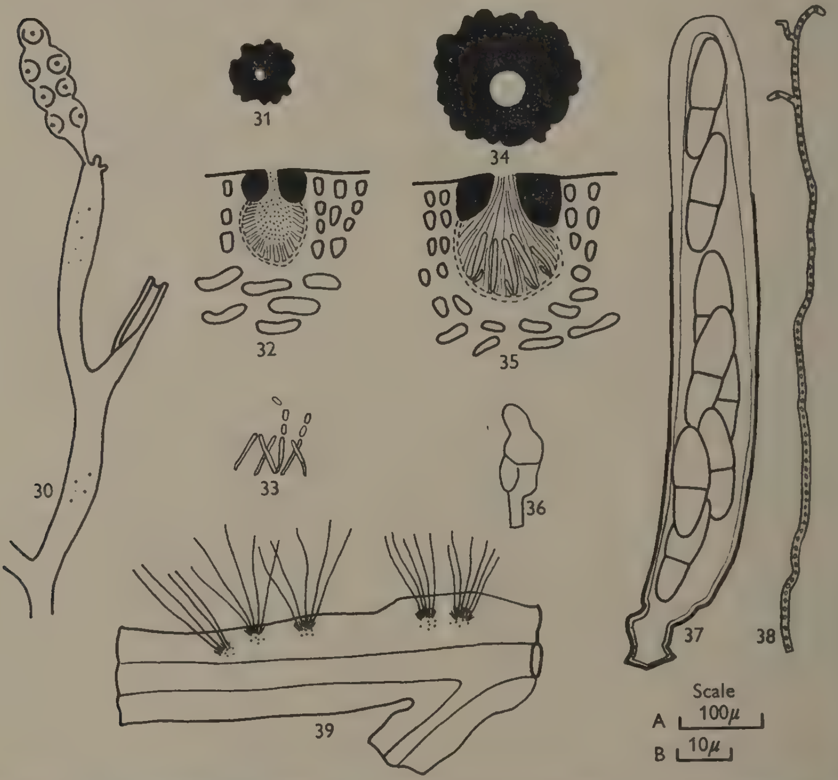
DIDYMOSPHAERIA PELVETIANA SUTH. ON *PELVETIA CANALICULATA* AND *D. FUCICOLA* SUTH. ON *FUCUS VESICULOSUS*

These two species were originally described (Sutherland, 1915) as parasitic, both hosts being also completely covered by an epiphytic growth of *Elachista*. Perithecia were described only for *D. pelvetiana*. They were immersed, surrounded by a black pseudo-clypeus, and contained simple or branched paraphyses and asci with eight, biseriate, bi-celled, faintly coloured ascospores. *D. pelvetiana* was found recently on tattered, regenerating plants of *Pelvetia* at Aberporth, Cardiganshire, and from this material some further information can be added to Sutherland's description (Figs. 30–38).

The perithecium is a pseudothecium in the modern sense, and in the young stage, vertical hyphae or pseudoparaphyses are present as in

D. danica (Fig. 15). The pseudothecia measure approx. 159 × 133 μ and are yellowish. The asci are bitunicate and the ascospores sometimes distinctly yellowish brown. The spores measure 19–23 × 4–6 μ, agreeing well with the figures given by Sutherland (Table 1).

In addition to ascocarps, pycnidia, 115–130 × 80–85 μ, were found, completely immersed in the convex side of the channelled frond, each surrounded by a black pseudo-clypeus and containing long, hypha-like conidiophores lining the inside of the wall. The conidia are hyaline, oblong and approx. 3 × 1 μ or less.



Figs. 30–38. *Didymosphaeria pelvetiana.* 30, pycnidia and ascocarps on convex surface of old, proliferating thallus of *Pelvetia canaliculata* (× 2); 31, pseudo-clypeus over pycnidium; 32, section of pycnidium; 33, conidiophores and conidia; 34, pseudo-clypeus over ascocarp; 35, section through ascocarp; 36, crozier; 37, bitunicate ascus, inner wall beginning to stretch upwards, containing eight bi-celled ascospores; 38, pseudoparaphysis; 31, 32, 34, 35, scale A; 33, 36, 37, 38, scale B. Fig. 39. *D. ? fucicola.* Pycnidia on thallus of *Fucus vesiculosus* with epiphytic *Elachista fucicola* (× 1).

Pycnidia of similar structure, but slightly larger, approx. 160 μ diam. and more globose, with a distinctly coloured wall, have been found on the thallus of *Fucus vesiculosus*, near epiphytic plants of *Elachista* (Fig. 39). It is thought that these may be the pycnidia of *Didymosphaeria fucicola*, probably observed by Sutherland but without conidia.

Table 1. *Comparison of* Didymosphaeria danica, D. pelvetiana *and* D. fucicola

(Measurements in μ.)

Name used	Authority	Pycnidium	Conidia	Perithecia	Asci	Ascospores
		D. danica on *Chondrus crispus*				
Leptosphaeria marina Rostr.	Rostrup, 1889	—	—	No measurements		Hyaline or pale yellow
L. marina Rostr. (non. Ell. & Ev. 1885)	Saccardo, 1891	—	—		50 × 10	16 × 4, hyaline or pale yellow
L. danica Berlese	Berlese, 1892	—	—	No measurements		
Sphaerella chondri Jones	Jones, 1898	—	—			
L. chondri (Rostr.) Rosenv.	Rosenvinge, 1906	156–175 × 100	4 × 1	125–215 × 100–300	75–80 × 11–13	33–34 × 5–6, hyaline
L. chondri (Rostr.) Rosenv.	Cotton, 1908	150–175 × 85–100	4 × 1	125–215 × 110–300		29–31 × 5–6, hyaline
Didymosphaeria marina (Rostr.) Lind	Lind, 1913	150–175 × 85–100	4 × 1	125–215 × 110–300	70–80 × 10–15 70–80 × 10–15	25–40 × 5–7, hyaline 25–40 × 5–7, brown
D. danica (Berlese) Wils. & Knoyle	Wilson & Knoyle	100–192 × 63–113	4 × 1	180–267 × 156–277	83–94 × 12–14	33–40 × 5–7, hyaline or pale yellow
		D. pelvetiana Suth. on *Pelvetia canaliculata*				
D. pelvetiana Suth.	Sutherland, 1915	—	—	90–115 diam.	55–75 × 9–11	18–24 × 5–6, faintly coloured
D. pelvetiana Suth.	Wilson & Knoyle	115–130 × 80–85	3 × 1	159 × 133 approx.		19–23 × 5–6, yellowish brown
		D. fucicola Suth. on *Fucus vesiculosus*				
D. fucicola Suth.	Sutherland, 1915	?	—	120–140 diam.	70–90 × 10–14	16–22 × 7–8, slightly coloured
D. ? fucicola Suth.	Wilson & Knoyle	160 diam.		—	—	—

D. pelvetiana is quite distinct from *D. danica*, but *D. fucicola* and *D. pelvetiana*, as pointed out by Meyers (1957), appear rather similar, though Sutherland considered them to be distinct because of the difference in spore shape. The absence of the perfect state in our material of *D. fucicola* did not allow this point to be clarified.

DISCUSSION

The three marine fungi considered here have some striking features in common. They are all parasitic in seaweeds with an intercellular mycelium in the host. They form immersed pycnidia surrounded by a pseudo-clypeus and containing long, hypha-like and apparently mainly unbranched conidiophores producing minute oblong, hyaline conidia. The immersed pseudothecia, which also have a pseudo-clypeus, contain numerous thread-like and occasionally branched pseudoparaphyses and bitunicate asci containing eight irregularly biseriate, bi-celled ascospores varying from hyaline to brown. The bitunicate asci and the ascostromatic fruit body clearly show that they belong to the Ascoloculares of Nannfeldt (1932), or the Bitunicatae or the Loculoascomycetes as this group has been called by later authors (Luttrell, 1951, 1955; Arx & Müller, 1954).

To place these fungi correctly in a genus is difficult and their suggested position in *Didymosphaeria* requires justification. Fuckel founded the genus in 1869, describing it as 'Corticolae. Perithecia ut Pleospora. Ascosporae didymae, fuscae.' The various ways in which the genus has been delimited by subsequent authors have been summarized by Scheinpflug (1958), but the two-celled, coloured spores remain an integral part of the definition in modern work.

Lind's contention that *D. danica* has two-celled spores has been fully confirmed in this investigation; all three fungi under consideration have this character. The spores of *D. danica* are usually hyaline, but slightly yellowish or even brown ones have been recorded (Table 1). Similarly *D. pelvetiana* and *D. fucicola* may produce slightly to distinctly coloured spores. The fact that the spores may become coloured, though the production of pigmentation is sporadic and the circumstances in which it appears are unknown, appears more significant than the fact that the spores are frequently hyaline. If this argument is not accepted and these species are not included in *Didymosphaeria*, there remain *Keissleriella* v. Höhn and *Didymella* Sacc., both with hyaline spores, as alternatives. The possibility of a permanently juvenile state of *Leptosphaeria* must also be considered, owing to the persistence of this idea in the literature.

Keissleriella agrees with *Didymosphaeria* in the structure of the asci, pseudoparaphyses and wall and in the formation of the clypeus (Scheinpflug, 1958), but in *Keissleriella* the ostiole projects and the pore is covered with dark brown bristles, a feature not seen in any of the three fungi under consideration. Moreover, most of the members are hypersaprophytes on rotten wood (Munk, 1957).

Didymella was separated from *Didymosphaeria* by Saccardo (1882) for those species with hyaline spores which had been included in the genus by Niessl in 1857, but recent studies (Arx, 1949; Corbaz, 1956) support the earlier view of Petrak (1923) that *Didymella* is more closely related to *Mycosphaerella* than to *Didymosphaeria*. The most obvious difference from *Mycosphaerella* is the retention of interascicular tissue at maturity and the parallel asci. Müller & Arx (1950) have pointed out differences in spore shape and Corbaz (1956) has stressed the difference in the imperfect form. *Mycosphaerella* has the *Dothidea*-type of development (Luttrell, 1951), but it seems unlikely that *Didymella*, with its pseudoparaphyses, does so too and therefore its relationship with *Mycosphaerella* may not be so close as was thought.

The imperfect form may well throw some light on the separation of genera; *Didymella* has pycnidia of the *Phoma*, *Phyllostictina* or *Diplodina*, *Ascochyta* type (Corbaz, 1956; Müller, 1958) with short or inconspicuous conidiophores and one- or two-celled, hyaline conidia. The imperfect states assigned to *Leptosphaeria* include the hyphomycetous genera *Alternaria* (Simmons, 1952; not confirmed by Müller, 1953) and *Cladosporium* (Müller, 1950), pycnidial types with large, coloured or hyaline and variously septate spores, such as *Camarosporium*, *Rhabdospora*, *Hendersonia*, *Phaeoseptoria* (Müller, 1950, 1952; Müller & Tomasevic, 1957; Webster, 1955*a*, *b*) and *Septoria* (Müller, 1952; Hughes, 1949), and pycnidial forms with small, one-celled conidia, such as *Asteromella* (Müller, 1950), *Coniothyrium* (Webster, 1955*a*), '*Phoma*-type' (Müller, 1953) and *Leptophoma* (distinguished from *Phoma* by Höhnel in 1915 by the rod-shaped conidia (Müller & Tomasevic, 1957)). The imperfect state of several species of *Didymosphaeria* belongs to *Dendrophoma* Sacc. (Müller & Corbaz, 1956; Scheinpflug, 1958), defined by Saccardo as a pycnidial form with verticillately or simply branched conidiophores (branches sometimes reduced to mere teeth), pointed at the apex, and with very small, hyaline, oblong or ovoid conidia.

The *long* conidiophores of the three marine fungi, ascribed here to *Didymosphaeria*, distinguish their pycnidia from those of *Didymella* and *Leptosphaeria*, but their *unbranched* condition does not fit with the known imperfect forms of *Didymosphaeria*. Nevertheless, they resemble more closely Scheinpflug's figures (1958) of the imperfect form of *D. futilis* with its feebly branched conidiophores than any of the imperfect states of *Didymella* or *Leptosphaeria*.

Sutherland's doubts about the inclusion of *Didymosphaeria pelvetiana* and *D. fucicola* in the genus, in so far as they were based on the presence of a clypeus, need no longer stand, since recent treatments of the genus (Müller & Arx, 1950; Scheinpflug, 1958) and even the restricted definition of Munk (1957) include both clypeate and non-clypeate types.

The position of *Didymosphaeria* within the bitunicate group has been in the Pseudosphaeriales, since that order was founded in 1918, and in the family Pleosporaceae, but Munk (1953) removed it to the Didymosphaeriaceae, and Luttrell (1955), accepting this family, placed it in the new order Pleosporales, distinguished by its *Pleospora*-type of development. Holm (1957) retains *Didymosphaeria* in the Pleosporaceae. In a critical consideration of the six species on which Fuckel's genus was founded he points out that *D. peltigerae* deviates biologically from the description of Fuckel, according to which the species are 'corticolae'. Such a very strict definition of the term, which was obviously not intended by the founder of the genus, would also exclude the species on algae, but *D. peltigerae* is retained in *Didymosphaeria* by Scheinpflug (1958).

In conclusion, from a new examination of the material and a comparison with other genera, we support the view of Lind (1913) that the fungus on *Chondrus* is best assigned to *Didymosphaeria*, but consider that it should be called *D. danica*, reviving the first valid specific epithet assigned to it by Berlese in 1892. *D. pelvetiana* and *D. fucicola* clearly belong to the same genus as *D. danica*.

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THE RHIZOMORPHS OF *MARASMIUS ANDROSACEUS* FRIES

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(With Plate 5)

Rhizomorphs were formed on the dikaryotic mycelium only. On agar slants they developed equally well whether the inoculum was mycelium or rhizomorph tip. To a limited degree only, the presence of rhizomorphs restricted the production of new rhizomorphs. They developed better on oatmeal- than on malt-, heather-stem-sawdust-, or heather-leaf-extract-agar. Initials were most numerous on this last agar, but subsequent development was limited. Mycelial growth was restricted and rhizomorph development inhibited when the pH value of the medium was as high as 7 or as low as 4. Over the range of temperatures from 35 to 15° C., 20° C. gave best development. Rhizomorphs developed freely in atmospheres with high relative humidity (76–89 %), but did develop also at very low relative humidity. Maturation of fructifications was restricted to cultures growing within the higher humidity range. Rhizomorphs responded negatively to gravity, and positively to light of sufficient intensity. Both mycelium and rhizomorph tips gave a positive reaction with Nobles's (1958) test for extracellular oxidase, indicating ability to attack lignin. Rhizomorphs did not tend to twine round glass rods or pieces of dead or living stems of several woody species of plants that were tested, but they did twine round and adhere to living stems of *Calluna vulgaris*. Under humid conditions, these living stems were penetrated, and the tissues of bark, wood and pith were invaded, within a period of 12 weeks.

Accounts of *Marasmius androsaceus* Fries have been published by Macdonald (1949) and Townsend (1954), dealing with various aspects of the life history, and by Braid & Tervet (1937), Macdonald (1948) and Braid & Lewis (1954), dealing with its parasitism of *Calluna vulgaris*.

Townsend (1954) in her account of fungus rhizomorphs stated that her cultures of *M. androsaceus* failed to produce any rhizomorphs. In this department, both pre-war and continuously since 1946, cultures of the fungus have produced rhizomorphs regularly and freely. Additional observations are now presented, to bring information available on them more into line with that published on *Armillaria mellea* by Garrett (1953) and Townsend (1954). Dennis & Gray (1954) have expressed the view that the parasitism of *M. androsaceus* on heather ought to be reinvestigated. Some supplementary experiments have, therefore, been carried out.

EXPERIMENTAL WORK

Type of medium. Malt-, oatmeal-, heather-stem-extract- and heather-leaf-extract-agar were tested. The heather agars were made by boiling leaves or finely ground stems for an hour, filtering and making up the filtrate with distilled water to the volume required to give a 2% decoction.

The oatmeal agar gave the most vigorous growth of mycelium and rhizomorphs. Rhizomorphs were longer, thicker and blacker than on malt agar. They appeared earliest and were most numerous on heather-leaf-extract agar, but did not maintain the same growth rate over a 7-week period as those formed either on oatmeal or on malt. This failure of an agar medium of low nutrient status to support continued growth of an initially large number of rhizomorphs has been observed in cultures of *Armillaria mellea* by Garrett (1953).

The apex of a young rhizomorph usually shows marked coiling (Pl. 5, fig. 1). This feature persists when growth is slow and the rhizomorph remains stunted. If considerable extension takes place, the coiling disappears. Branching occurs frequently, the incipient branches appearing as bud-like swellings well behind the rhizomorph apex (Pl. 5, fig. 2).

Type of container and depth of medium. Cultures were grown in test tubes, boiling tubes, Petri dishes and conical flasks. With the dishes and flasks, various depths of medium were tried. Deeper layers of medium appeared to increase the numbers of rhizomorphs produced.

Type of inoculum. Campbell (1934) transferred to fresh media tips of rhizomorphs of *Armillaria mellea* which had developed in culture. These tips continued to grow as rhizomorphs and even developed branches. No aerial mycelium developed in such cultures on malt agar. Rhizomorphs of *M. androsaceus* did not behave in this way. Mycelium or rhizomorph tips transferred to agar slants both gave rise to a fan of hyphae spreading out from the inoculum. After 2 months these cultures were indistinguishable in their growth type and both formed rhizomorphs. Garrett (1953) reported that in *Armillaria mellea* growing on a medium of adequate nutrient status there was suppression of rhizomorph formation by those rhizomorphs already present round the inoculum. This suppression he considered to be a result of the depletion of nutrients from the medium due to their removal by existing rhizomorphs. This suppression effect is more restricted in *M. androsaceus* as rhizomorphs may form on any part of the surface of agar slants in addition to the area occupied by the inoculum. There is, however, a marked tendency for them to form more freely towards, and even be restricted to, the foot of the slant, i.e. to that part furthest removed from the inoculum and at which the deepest layer of medium occurs. In plate and flask cultures in which the medium was of uniform depth and in which the inoculum was placed as near the middle as possible, rhizomorphs formed in a series of concentric rings, showing a definite though more localized suppression effect (Pl. 5, figs. 3 and 4).

pH *of medium.* Rhizomorphs were found growing very freely among the moss *Pleurozium schreberi* with a large number of needles of *Pinus nigra* on a Forestry Commission site at Tentsmuir, 5 miles north of St Andrews. Samples of the soil were collected and the pH estimated to be 4·2, using a Universal pH meter and millivoltmeter, with a buffer solution pH 4 for standardizing.

Oatmeal and malt agars were used in experiments lasting 8 weeks to test the effect of different pH values on rhizomorph development in culture. With oatmeal agar it proved very difficult to make the adjustments of pH, because large quantities of liquids had to be added to overcome the strong buffering of this medium.

Table 1. *Effect of* pH *of medium on mycelial growth and rhizomorph production by* Marasmius androsaceus

	pH	Adjusted with	Growth of (a) Mycelium	 (b) Rhizomorphs
Oatmeal agar	7·0	N-NaOH	Nil	Nil
	6·2	NaCl with equivalent Na to above	Nil	Nil
	6·2	Unadjusted	Profuse	Formed
	5·0	N-HCl	Profuse	Formed
	4·0	N-HCl	Very reduced	Nil
Malt agar	7·0	N-NaOH or N-KOH	Very reduced	Nil
	6·4	NaCl or KCl with equivalent Na or K to above	Good	Formed
	6·4	Unadjusted	Good	Formed
	5·0	N-HCl	Good	Formed
	4·0	N-HCl	Reduced, but better than at pH 7·0	Nil

Temperature. Experiments lasting 40 days and upwards were carried out with oatmeal and malt agar slants incubated in darkness at 35, 30, 25, 20 and 15° C. Of the temperatures tested, 20° C. gave the best growth of mycelium and the most numerous and longest rhizomorphs on both media. There was no formation of rhizomorphs at 35 and 30° C. Representative figures for total spread of the mycelium up and down from the inoculum over the surface of the slants after 40 days were: 35° C., 3·1 cm.; 30° C., 4·7 cm.; 25° C., 7·5 cm.; 20° C., 9·8 cm.; 15° C., 7·0 cm.

Humidity. Four sets of agar slants were put in a wooden frame covered with glass lights. The frame was constructed within a glasshouse and contained a layer of peat approx. 15 cm. deep. Three of the sets were placed under belljars. In two, the relative humidity of the atmosphere was adjusted to and maintained as nearly as possible at 1 and 56 %, using anhydrous calcium chloride and a solution of technical calcium nitrate, respectively. In the third the range was not adjusted and varied from 85 to 89 %. The fourth set was kept uncovered in the frame. The relative humidity varied from 76 to 88 %. The surface of the peat was kept moist, except under the two belljars in which the relative humidity was adjusted. After 28 days, rhizomorphs had formed in all cultures in each of the four sets of slants. They were more numerous and longer on average under the high humidities.

Gravity. Macdonald (1949) showed that rhizomorphs were negatively geotropic, but an experiment in the present series suggested that the response might be restricted to young rhizomorphs. Petri-dish cultures over 3 months old, containing rhizomorphs which were growing vertically upwards, were covered with black paper and placed on their sides for a further 3 weeks. The rhizomorphs then showed a somewhat random direction of growth. The possibility that this might be due to slight light leaks in the paper could not be discounted. To clear this point up, slants were inoculated with mycelium only and placed immediately in total darkness in closed tins. They were divided into four lots: (*a*) with the agar surface vertical and the mouth of the tube upwards, (*b*) as in (*a*) but with the tube mouth down, (*c*) with the tube horizontal and the inoculated surface of the slant upwards, (*d*) as in (*c*) but with the inoculated surface down. The cultures were examined after 3 months. In sets (*a*), (*b*) and (*c*) the rhizomorphs all grew vertically upwards. In set (*d*) they grew either pressed against the surface of the medium throughout their length or with their tips at least pressed against it. The tubes of (*a*) were now placed with the mouth downwards and those of (*b*), (*c*) and (*d*) were also reversed in position. After a further 3 months in darkness, the direction of all new growth again showed a negative geotropic response.

These experiments confirm that both young and old rhizomorphs are negatively geotropic. On many occasions they obtain support by twining round each other. They still grow vertically upwards in these circumstances. The possibility that direction of growth is being influenced by unequal concentration of oxygen or other gas is excluded by the results of treatment (*b*), in which the direction of growth is away from the open end of the tube.

Gravity and light. Four sets of tubes were grown in darkness for 3 months, as described in the gravity experiments. One half of the tubes from each was then placed in a glass fronted incubator in the same relative positions as previously. These were exposed to natural light and darkness for 3 months, during the autumn–winter period from 13 October 1959 to 15 January 1960. The incubator did not receive any direct sunlight and the reading obtained with a Weston III Exposure Meter at no time exceeded 10. The other half-sets of tubes remained in darkness in the closed tins in the same positions. At the end of the second 3 months there was no change in the established direction of growth of the rhizomorphs, either in light or in darkness. Under the conditions of the experiment, gravity proved a more powerful stimulus than did light of low intensity. To test if light of higher intensity would prove a more powerful stimulus than gravity, a set of four 15-week-old Petri-dish cultures, with vertically growing rhizomorphs, was used. These were laterally illuminated through slits with daylight supplemented by light from a 200 W. electric lamp, directed towards the slits from a distance of 3 ft. This combination of sources of light gave readings with the Weston Mark III Meter which varied from 50 to 200 over the same period as in the previous experiment. The tips of all rhizomorphs on the side of the Petri dishes nearest to the slits bent over in the direction of the incident light. Rhizomorphs in other parts of the dishes were not visibly affected. This confirms that a minimum intensity of light is necessary to produce a directional stimulus to growth.

Ability of M. androsaceus *to attack lignin.* Macdonald (1949) showed that the fungus in malt agar culture with 0·5 or 0·25 % tannic acid gave oxidation rings. This is the Bavendamm (1928) test which was accepted by Davidson, Campbell & Blaisdell (1938) as indicating a lignin-attacking species. Nobles (1958) obtained agreement with results obtained by the

Bavendamm method for nearly 90 % of species when she tested for the presence of an extracellular oxidase by applying an alcoholic solution of gum guaiacum directly to cultures of species of wood-inhabiting hymenomycetes.

This latter test was applied to 3-week-old cultures on agar slants, to rhizomorph tips and to chopped up pieces of mature rhizomorphs. Strong blue coloration developed with the young mycelium and with the rhizomorph tips but not with the old rhizomorphs, showing that the actively growing mycelium and young rhizomorphs produced extracellular oxidase. This confirms that *M. androsaceus* comes into the group of fungi associated with the destruction of lignin and the consequent production of white rots.

Absence of luminosity. *Armillaria mellea* is well known as a fungus which renders attacked wood luminous and luminosity is exhibited also by the rhizomorphs. *M. androsaceus* does not exhibit luminosity in nature or in culture, whether it is present in the form of rhizomorphs, mycelium or fructifications.

Absence of rhizomorphs from monospore culture. Rhizomorphs have not appeared in any of the numerous monospore cultures which have been maintained, in some cases for years, in this Department. Their appearance is linked with the presence of clamp connexions on the hyphae and they are restricted to dikaryotic mycelia.

Interrelationship of rhizomorphs, fructifications and pseudosclerotia. Fructifications were formed at the two high humidities used in the relative humidity experiments. The stalks of these fructifications were not distinguishable from rhizomorphs until 8 days before the development of pilei became obvious. From then on, the stalks were markedly thicker and sometimes flattened, but did not differ otherwise from rhizomorphs in their morphology (Pl. 5, fig. 5). The fructifications produced spores. When moistened after drying, they produced fresh spores in the manner characteristic for the genus.

Macdonald (1949) has drawn attention to the close similarity in appearance and structure between naturally occurring rhizomorphs and fructification stalks. It now seems that high relative humidity is necessary for fructification formation though not for rhizomorph development. In nature, fructifications of *Armillaria mellea* develop attached to rhizomorphs. That is to say, there is a close physical relationship in that species also.

A close relationship exists between the formation of rhizomorphs and pseudosclerotia delimited by black lines in plant tissues. Black lines form in cultures of *M. androsaceus*. They are sometimes clearly associated with the bases of rhizomorphs. When rhizomorphs are pulled away from cultures part of the black line attached to the rhizomorph base may also come away. The wall or limiting layer structure of this extension of the rhizomorph is shown in Pl. 5, fig. 6. This supports the view that the black line is the wall of a sclerotium embedded in the medium, from which the rhizomorph arises.

Adhesion and twining of rhizomorphs. The rhizomorph has adhesive properties as long as it is moist. This was shown by placing dry and moistened rhizomorphs on glass slides. The former did not adhere to the glass whereas the latter did.

A 4-in. length of sterilized woody stem of *Erica stricta* was introduced into each of five conical-flask cultures containing well-established mycelium and vertically growing rhizomorphs. A sterilized glass rod was introduced into a sixth similar culture. After 5 weeks, those rhizomorphs which had come into contact with the stems and the glass rod showed no tendency to twine round these objects. Observations at intervals for 6 months confirmed this.

Potted specimens of three woody plants—*Veronica* × *lindsayii*, *V. pimelleoides* and *Hypericum fragile*, which happened to be available, were placed under humid conditions on peat in a glasshouse frame and were watered frequently to encourage free development of rhizomorphs. Pieces of an oatmeal agar culture with numerous rhizomorphs were buried in the surface layers of the soil in each pot. After 6 weeks there was some further development of rhizomorphs, but they showed no tendency to twine round the stems of any of the plants. This experiment was repeated on two other occasions, with the same result.

Six potted plants of *Calluna vulgaris* were set up under the conditions described above. One was used as a control. Mycelium with rhizomorphs was placed in the soil of the other five pots. Further development of rhizomorphs occurred in all five pots. Some reached lengths up to 14 cm. The longer rhizomorphs twined round heather stems and showed some points of close contact. The tips were pale in colour, indicating continued rapid growth at the apex region. This experiment was repeated with two more heather plants and gave the same type of result. At the points of contact with the stems, rhizomorphs sometimes adhered firmly. Fans of white hyphae could be seen emerging from the rhizomorphs, spreading over the stems and apparently penetrating them.

Infection experiments. Three plants of *Calluna vulgaris* in pots were placed on a deep layer of peat inside larger pots, so that the rims of the two pots were at the same level. The space between the inner and outer pots was packed with the moss *Pleurozium schreberi* and a very dense layer of the moss was placed on the surface of the soil. The moss contained numerous needles of *Pinus nigra* carrying rhizomorphs of *M. androsaceus*. Plants were placed under a cheese-cloth cage in the glasshouse and were watered daily. After 1 week, rhizomorphs were seen growing among the bushy stems of the heather plants. After 8 weeks, they were up to 19 cm. in length, twining round and adhering to the stems. At several of the points of contact, fans of white hyphae were seen emerging from the rhizomorphs and spreading over the stem surfaces.

Pieces of living heather stem with attached rhizomorphs produced in this experiment were cut after 12 weeks and stained with safranine and picro-aniline blue by Cartwright's (1929) method. Transverse and longitudinal sections showed hyphae in the dead and living bark, and also in the wood and pith. There were no hyphae to be seen in similarly prepared sections from the stem of the control plant.

Quite heavy concentrations of hyphae were present in the bark at the point of contact with the rhizomorph. The amount diminished with increasing distance away from this point. It seems clear, therefore, that the rhizomorph was the means whereby the stems became infected. This agrees with Macdonald's (1949) observations on natural infections. What is remarkable is the rapidity with which the fungus invaded all stem tissues during experiments lasting only 12 weeks, and the absence of any obvious reaction by the host to the parasite.

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EXPLANATION OF PLATE 5

Fig. 1. Circinate coiling of rhizomorph tip. × 75.

Fig. 2. Bud-like swelling on rhizomorph, due to young branch. × 75.

Fig. 3. Malt-agar Petri dish culture showing inoculum near middle of plate and three more or less concentric regions with partial rings of rhizomorphs. × 2/3.

Fig. 4. A culture similar to that of Fig. 3, with a single ring of rhizomorphs and the beginning of a second ring at the plate edge. × 2/3.

Fig. 5. Tube culture with rhizomorphs and fructifications. × 5/6.

Fig. 6. Wall-like arrangement of black line cells attached to base of rhizomorph. × 75.

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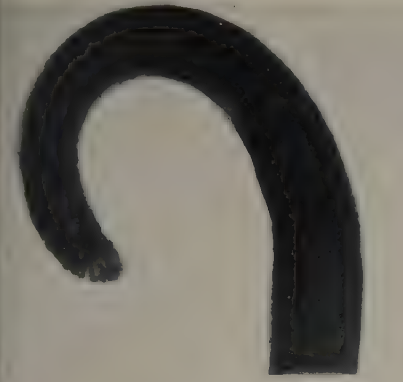

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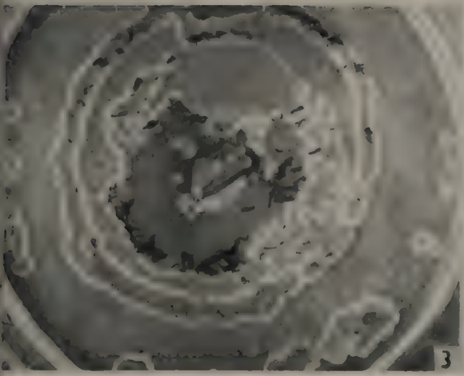

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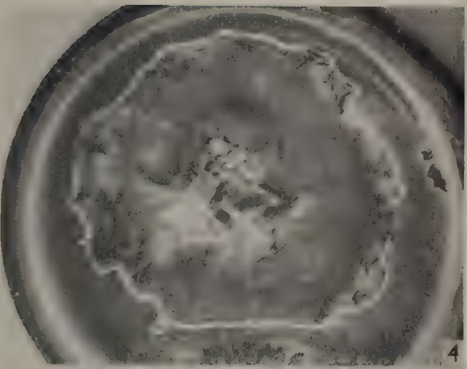

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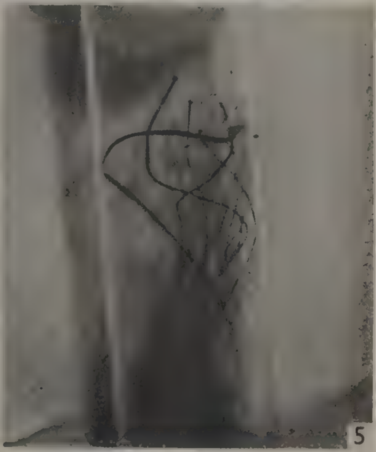

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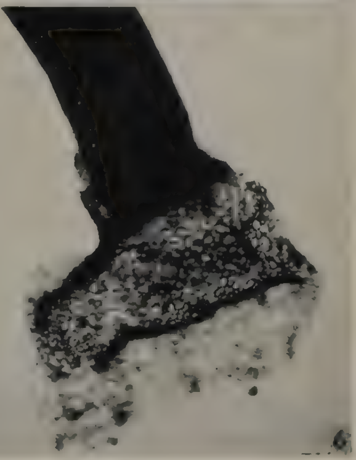

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CHAETOMIUM PILULIFERUM SP.NOV., THE PERFECT STATE OF *BOTRYOTRICHUM PILULIFERUM*

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(With Plate 6 and 3 Text-figures)

A culture of *Botryotrichum piluliferum* Sacc. & March. isolated from cellulose film previously buried in soil, produced perithecia typical of *Chaetomium* in addition to aleuriospores, phialospores and dark hyphae characteristic of *B. piluliferum*. Ascospores were germinated and formed colonies of *B. piluliferum*. The perithecia were not identified with any known species of *Chaetomium*, and the fungus is diagnosed as *C. piluliferum* sp.nov.

The identity of *Chaetomium* and *Botryotrichum* was observed while studying cellulose-decomposing fungi in sandy field loam from the University Farm, Cambridge, when perithecia of *Chaetomium* grew from a culture of *B. piluliferum* Sacc. & March. The relationship had been suspected earlier by Tribe (1957, 1958) who found fungi which, although they could not be identified completely with either *Botryotrichum* or *Chaetomium*, nevertheless, possessed some characteristics of both genera. On buried cellulose film these fungi produced phialospores and rooting branches similar to those of *B. piluliferum* and immature perithecia similar to those of *Chaetomium*. However, they formed neither the aleuriospores typical of *B. piluliferum*, nor mature perithecia of *Chaetomium* on either cellulose film or potato dextrose agar, but their relationship with *Chaetomium* was indicated when ascospores of *C. elatum* formed colonies identical with them. The new isolate is compared with a closely related species of *Chaetomium* and with *B. piluliferum*.

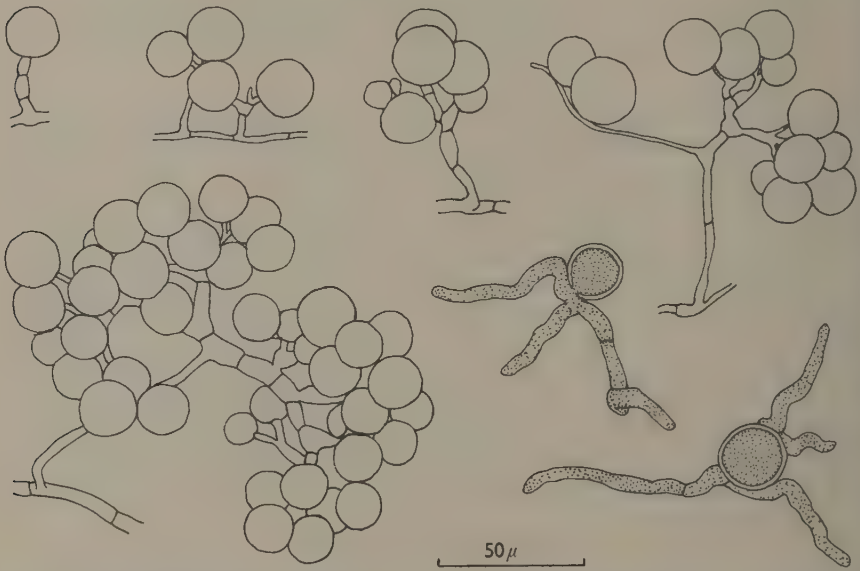
METHOD OF ISOLATION

By means of a technique similar to that described by Tribe (1957), fungi were isolated into pure culture from washed cellulose film previously buried in soil. Pieces of the film 7 × 2 cm. were placed on clean microscope slides which were then inserted vertically into the soil at 60 % water-holding capacity and incubated at laboratory temperature (approx. 17° C.). After 10 days the film showed fungal hyphae both etching the surface and forming rooting branches inside the film (Pl. 6, figs. 1, 2), details of which are given by Tribe (1957). The film was washed in tap water, pieces 2 mm. square were plated on potato dextrose agar containing 0·03 % streptomycin and, after incubation overnight at 25° C., hyphae were often seen growing from the etchings or from the rooting branches. They were subcultured immediately and, on all occasions, a high proportion was identified as *B. piluliferum*. Two of these isolates, when incubated for 3 weeks, formed *Chaetomium* perithecia in addition to *Botryotrichum* aleuriospores.

MORPHOLOGY OF THE FUNGUS IN CULTURE

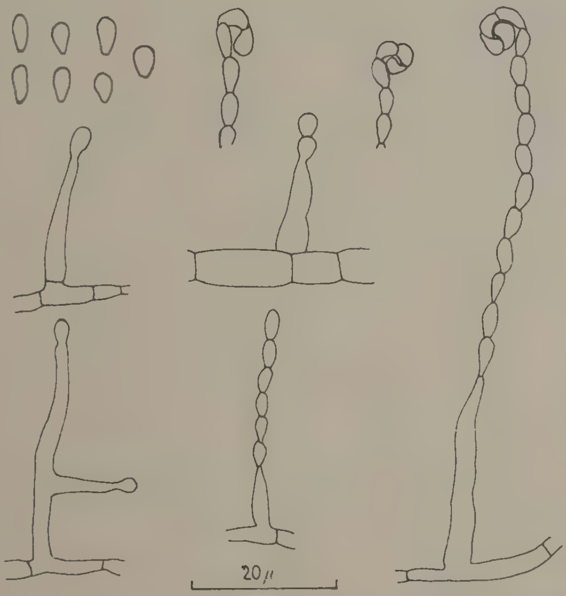
Imperfect and perfect states

The morphological observations were made from a culture growing on potato dextrose agar and measurements from living material mounted in water. Aerial and intramatrical mycelium was abundant and consisted mostly of hyaline septate hyphae together with some dark brown, septate hyphae which are referred to by Downing (1953) as 'sterile hyphae'. The dark brown hyphae were aerial and/or superficial and formed after about 8 days at 25° C. They were infrequently branched, ranged from 1·8 to 5·9 μ diam., were variable in length, and imparted a grey to black coloration to the colony. The hyaline hyphae were branched, ranged from 1 to 6 μ diam., and bore aleuriospores abundantly. The aleuriospores were hyaline, globose, averaging 14·2 μ diam. (standard deviation 0·8 μ), with walls up to 3·5 μ thick. They were borne singly on the intramatrical hyphae and either singly or in clusters on the aerial hyphae. In water or on agar media they germinated readily by one or more germ tubes (Text-fig. 1). Phialides and phialospores were not seen on potato dextrose agar but formed abundantly on glass coverslips of slide cultures of Czapek–Dox agar and also on the surface of sucrose-free Czapek–Dox agar. The phialides were hyaline, 10–30 μ long × *ca.* 5 μ broad, occasionally branched, and usually arose at right angles from the hyphae. Phialospores were hyaline, clavate, averaging 3·8 μ long (standard deviation 0·5 μ) × 2·2 μ broad at their greatest breadth, and formed in single chains of up to about



Text-fig. 1. Aleuriospores of *C. piluliferum* growing on aerial mycelial mycelium, and two spores germinating on potato dextrose agar. (From unstained material in water.)

35 spores. The chains were sometimes curled at the tips where the spores then often formed a cluster (Text-fig. 2). Perithecia began to form from superficial and aerial mycelium after 3 weeks at 25° C. (Pl. 6, fig. 3). One week later many were mature (Pl. 6, fig. 4) and clearly belonged to *Chaetomium*. They were globose to subglobose, opaque, 330–400 μ diam., and possessed a black, carbonaceous wall. The terminal and lateral perithecial hairs were dark brown and septate. Terminal hairs were 0·5–1·5 mm., long, averaged 4·7 μ broad (standard deviation 1·1 μ), and possessed open circinate, blunt tips. Lateral hairs were up to 1 mm. long and tapered gradually from 4·1 μ broad (average standard deviation 0·3 μ) at the base to 1·9 μ (average: standard deviation 0·4 μ) at the hyaline tips.

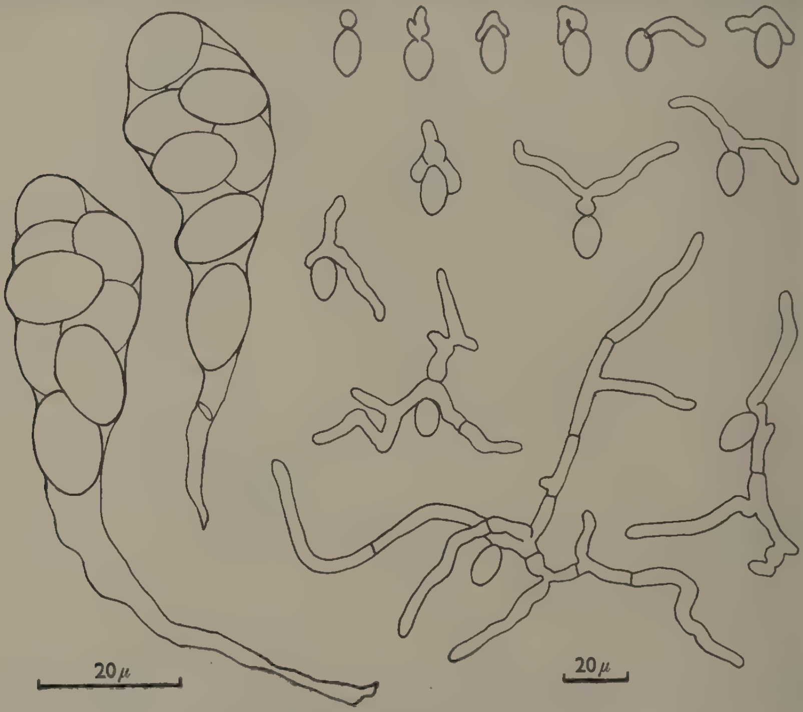


Text-fig. 2. Phialides and phialospores of *C. piluliferum* growing on sucrose-free Czapek–Dox agar. (From unstained material in water.)

Asci were irregularly clavate, approximately 60 μ long × 20 μ broad with an evanescent wall. Ascospores were brown, ellipsoid and averaged 14·8 μ long (standard deviation 0·6 μ) × 9·2 μ broad (standard deviation 0·7 μ). For comparison with dimensions given by Skolko & Groves (1953), they were also measured after warming in lactophenol, but found to be unchanged. When the ascospores were plated on to potato dextrose agar and incubated at 25° C. a few germinated by a terminal germ tube (Text-fig. 3). Twenty-two colonies derived from single ascospores were examined and all formed the aleuriospores and dark brown hyphae characteristic of *B. piluliferum*, but none formed perithecia. The dark brown hyphae and perithecial hairs were often roughened with hyaline, water-soluble material (Pl. 6, fig. 5), particularly when old.

Variation among the isolates

Of about 50 cultures similar to the above, isolated from cellulose in soil, only two possessed a full complement of morphological characters, including perithecia. Furthermore, the frequency of occurrence of dark brown hyphae, aleuriospores, phialospores and perithecia varied between extremes, so producing a wide range of colony characteristics. The dark hyphae occurred both as normal aerial mycelium and in discrete tufts, and ranged from olive green to brown and almost black. Cultures without dark hyphae were white. Two other cultures were distinguished by producing a pink coloration in the agar in addition to aleuriospores. Sectoring and annular zonation of colonies with respect to the presence or absence of dark hyphae also occurred.



Text-fig. 3. Asci and germinating ascospores of *C. piluliferum*. (From unstained material in water.)

A smaller range of variation occurred between 22 cultures from single ascospores than between the isolates from cellulose film. The ascospore mycelia were sparse and produced aleuriospores and a scattering of dark brown hyphae, with the exception of one culture which was blackened by a superficial layer and tufts of dark brown hyphae. The cultures were grown in pairs at random on potato dextrose agar and after 5 weeks incubation at 25° C. perithecia had not formed either within the colonies or along their lines of contact.

IDENTIFICATION

Direct comparisons were made between the new isolate and named cultures of closely related species of *Chaetomium* and *Botryotrichum*. In possessing ellipsoid ascospores and unbranched perithecial hairs with open circinate tips, the perfect state resembled most closely *C. murorum* Corda and it was compared with a culture and herbarium specimens of *C. murorum* from the Commonwealth Mycological Institute and with the descriptions of *C. murorum* by Chivers (1915) and Skolko & Groves (1953). Although the similarities with *C. murorum* were confirmed, the new isolate was found to differ clearly in a number of characters which may be summarized as follows. The ascospores were significantly longer and broader than those of all the isolates of *C. murorum* at the 0·001 level of probability (Table 1). The perithecial hairs of the new isolate (Pl. 6, fig. 4) are straighter and shorter than those of *C. murorum* I.M.I. 36633 (Pl. 6, fig. 6). Dark brown hyphae were abundant in the aerial mycelium of the new isolate but were absent in both the living culture of *C. murorum* I.M.I. 68391, and in a dried agar culture, I.M.I. 36633. Furthermore, the new isolate produced aleurospores and phialospores, whereas neither these nor other imperfect states have been reported in *C. murorum*.

Table 1. *Ascospore dimensions in μ of named isolates of* Chaetomium murorum *and the new isolate of* Chaetomium

		Length		Breadth		No. of spores measured
		Mean	Range	Mean	Range	
C. murorum ex Chivers, I.M.I. 34676	Prepared slide	13·2	11·8–14·7	7·6	6·6–8·8	30
C. murorum ex Warcup, I.M.I. 36633	Prepared slide	13·1	11·8–14	7·1	5·9–7·4	30
C. murorum I.M.I. 68391	Living culture	13·2	11·8–14·7	7·6	7·4–8·1	30
C. murorum ex Chivers (1915)	Description	12·6	11·3–12·9	8·1	—	—
C. murorum ex Skolko & Groves (1953)	Description	—	11–14	—	7–8	—
New isolate of *Chaetomium*	Living culture	14·8	13·2–16·6	9·2	8·8–10·3	30

The imperfect state of the new isolate was compared and matched satisfactorily with a culture of *B. piluliferum* from the collection of Dr H. T. Tribe and with the diagnosis of *B. piluliferum* by Downing (1953).

The perfect state of a *Botryotrichum* has not been reported previously, but imperfect states of species of *Chaetomium* have been described in a few instances. Thus phialides and phialospores, similar to those first described for *B. piluliferum* by Downing (1953), were recorded in *C. elatum* by Bainier (1909) and in *C. globosum* by Mason (1933), who figures an illustration by Zopf. Aleuriospores and intercalary chlamydospores were reported by Omvik (1955) in *C. homopilatum*. The aleuriospores were yellow-brown and either globose, measuring 6·3–8·4 μ diam., or oval and 8·4 × 6·3 μ, and so differed from the aleuriospores of the new isolate which were globose, hyaline and measured 14·2 (7·4–20) μ diam. *C. seminudum* was found by Ames (1949) to produce chlamydospore-like bodies 10–15 μ diam., which were usually borne on short, slender stalks, and also hair-like structures resembling perithecial hairs scattered over the surface of the agar. In all respects other than these, *C. elatum*, *C. globosum*, *C. homopilatum* and *C. seminudum* were quite different from the new isolate.

The foregoing comparisons showed that the perfect state of the new isolate was distinct from any known species of *Chaetomium*.

Chaetomium piluliferum sp.nov.

Mycelium abundant, intramatrical and aerial, composed of septate, hyaline or dark brown hyphae; hyaline hyphae 1–6 μ diam., intramatrical or aerial; brown hyphae 1·8–5·9 μ diam., superficial or aerial, infrequently branched, roughened with hyaline, water-soluble material when old. *Perithecia* 330–400 μ diam., globose to subglobose, with a black, carbonaceous wall. *Perithecial hairs* terminal and lateral, brown, septate and roughened like the brown hyphae when old. *Terminal hairs* 0·5–1·5 mm. long, 4·7(4·4–5·1) μ broad, cylindrical with open circinate, blunt tips. *Lateral hairs* up to 1 mm. long, tapering from 4·1(3·7–4·4) μ broad at the base to 1·9 (1·5–2·6) μ broad at the hyaline tip. *Asci* 60 × 20 μ, irregularly clavate with evanescent walls. *Ascospores* 14·8 × 9·2 (13·2–16 × 8·8–10·3) μ, brown, ellipsoid. *Aleuriospores* 14·2(7·4–20) μ diam., hyaline, smooth, with walls up to 3·5 μ thick, formed singly on the intramatrical hyphae, and singly or in clusters on the hyaline aerial hyphae. *Phialides* 10–30 × 5 μ, hyaline, occasionally branched and arising at right angles from the hyaline hyphae. *Phialospores* 3·8 × 2·2 (2·9–4·4 × 2·2) μ, clavate, hyaline, smooth, in single chains sometimes curled at the tips. Fungus very variable in production of brown hyphae, phialides and perithecia. Imperfect state *Botryotrichum piluliferum* Sacc. & March. A dried culture has been deposited in Herb. I.M.I. (No. 83238) as the type specimen.

Chaetomium piluliferum sp.nov.

Mycelium copiosum, intramatricale et aerium, hyphis septatis hyalinis vel obscure brunneis compositum; hyphae hyalinae 1–6 μ crassae intramatricales vel aeriae; hyphae brunneae 1·8–5·9 μ crassae, superficiales vel aeriae, sparsim ramosae, in aetate granulis hyalinis in aqua solubilibus asperatae. Perithecia 330–400 μ lata, globosa vel subglobosa, nigra, carbonacea, chaetis terminalibus et lateralibus brunneis septatis, ut hyphis brunneis in aetate asperatis, praedita: chaetae terminales 0·5–1·5 mm. longae, 4·7 (4·4–5·1) μ latae, rectae cylindricae apicibus obtusis apertocircinatae: chaetae laterales ad 1 mm. longae teretes, basi 4·1 (3·7–4·4) μ latae, ad apicem hyalinum 1·9 (1·5–2·6) μ latum attenuatae. Asci 60 × 20 μ irregulariter clavati, tunicis diffluentibus. Ascosporae 14·8 × 9·2 (13·2–16·6 × 8·8–10·3) μ, brunneae, ellipsoideae. Aleuriosporae 14·2 (7·4–20) μ latae, globosae, hyalinae, laeves, tunicis ad 3·5 μ crassis praeditae, ad hyphas intramatricales singulae, ad hyphas hyalinas aerias singulae vel plures glomeratae, raro intercalares. Phialides 10–30 × 5 μ, hyalinae, sparsim ramosae, ex hyphis hyalinis angulo recto orientes. Phialosporae 3·8 × 2·2 (2·9–4·4 × 2·2) μ, clavate hyalinae, laeves, in catenulis apicem versus aliquando curvatis uniseriatae. Species in productu hypharum brunnearum phialidum et peritheciorum multum variabilis. Status imperfectus Botryotrichum piluliferum Sacc. et March. est. Typus culturus siccus I.M.I. 83238 est.

DISCUSSION

C. piluliferum closely resembles other species of the genus in its history of powerful cellulolytic activity reported under the name *B. piluliferum*. It has been found on cellulosic substrates such as dung by Saccardo & Marchal (1885), on flypaper by Blochwitz (1914), on sacking by Wakefield (1919) and in soil in Canada by Bisby, James & Timonin (1933). There are few reports other than these, but the fungus may be more common than this evidence suggests for, though it is not a frequenter of soil dilution plates, it was often found by Tribe (1957, 1958) and during the work described here, decomposing cellulose in sandy field loam from the University Farm, Cambridge, and in sandy loam from Hildersham, near Cambridge. *C. piluliferum* is unusual in inhabiting soil, for the majority of *Chaetomium* spp. have been found above soil level; of about 60 species described up to the present, only 17 were listed by Gilman (1957) as occurring in the soil.

It is interesting to compare the relative occurrence of the perfect and imperfect states among species of *Chaetomium* usually found above soil level with the relative occurrence of these states of *C. piluliferum*. Above soil level perithecia predominate and many species appear unable to form an imperfect state. In *C. piluliferum*, on the other hand, the aleuriospore form predominates and only two out of about 50 isolates were able to form perithecia. The lack of development of the perfect state in culture was shown by other *Chaetomium*-like fungi found on cellulose in soil by Tribe (1957, 1958) when they formed phialospores and young perithecia which could not be induced to mature.

It might be expected that natural selection within species of *Chaetomium* inhabiting the soil would favour the development of characters different from those which would be favoured in species of *Chaetomium* living above soil level. In *C. piluliferum* and probably in other species of *Chaetomium* too, the soil appears to have favoured the development of the imperfect states almost to the exclusion of the perfect. Perhaps this is an instance of divergent evolution leading to an ecological separation of the perfect and imperfect states.

I am indebted to Dr H. T. Tribe for helpful suggestions and advice throughout this work and to the Director of the Commonwealth Mycological Institute for the generous loan of herbarium specimens. I should like to thank Dr E. J. H. Corner for kindly preparing the Latin diagnosis. My thanks are also due to Prof. Sir Joseph Hutchinson for providing laboratory facilities and to the Agricultural Research Council for financial support.

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EXPLANATION OF PLATE 6

Fig. 1. Rooting branches of *C. piluliferum* within the thickness of cellulose film previously buried in soil. Stained with picronigrosin in lactophenol and mounted in lactophenol (×60).

Fig. 2. The same (×190).

Fig. 3. Young perithecia and a few aleuriospores of *C. piluliferum* stained with picronigrosin in lactophenol and mounted in lactophenol (×70).

Fig. 4. Mature perithecium of *C. piluliferum* unstained and mounted in lactophenol (×35).

Fig. 5. Dark brown hyphae of *C. piluliferum* roughened with water-soluble material. Specimen unstained and mounted in glycerol. (×525).

Fig. 6. Mature perithecium of *C. murorum* Corda, Herb. I.M.I. No. 36633. Permanent preparation made by Warcup (×35).

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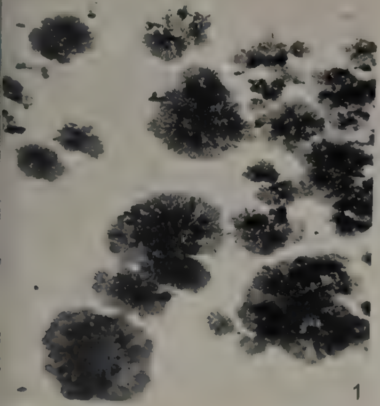

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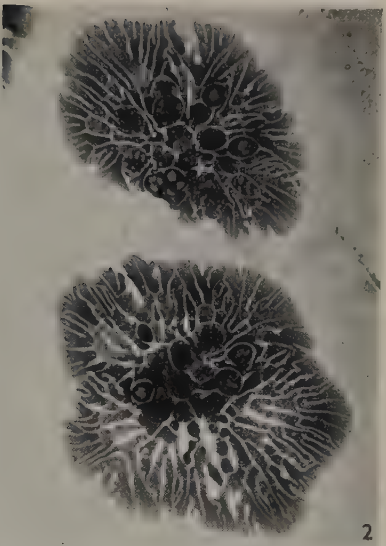

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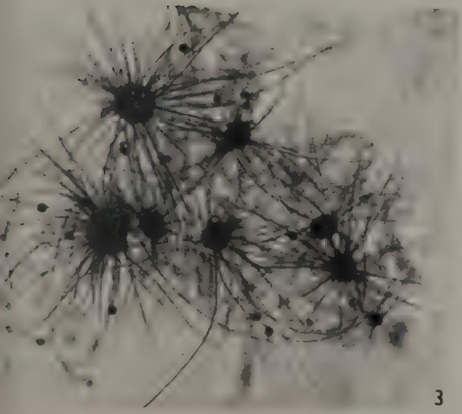

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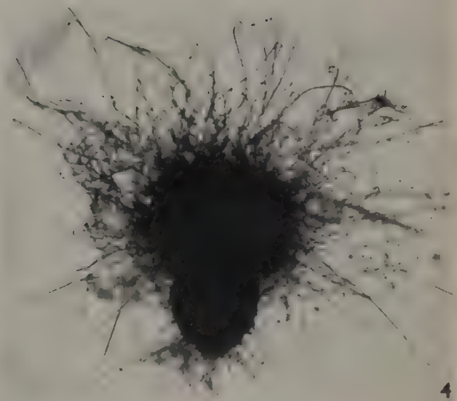

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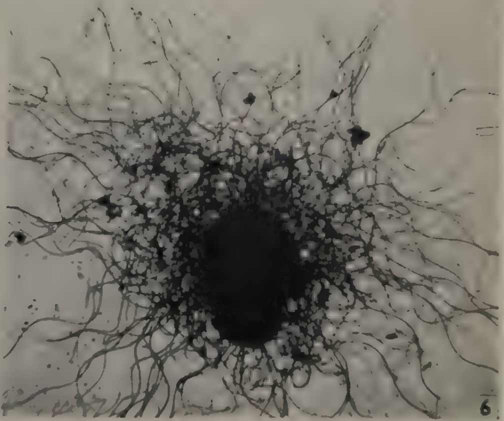

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Trans. Brit. mycol. Soc. **44** (1), 87–90 (1961).

STAGONOSPORA MACROPYCNIDIA SP.NOV.

By G. J. CUNNELL

Birkbeck College, University of London

(With 2 Text-figures)

Stagonospora macropycnidia sp.nov., found on dead leaves of *Carex pseudocyperus* L., *C. acutiformis* Ehrh. and *Typha latifolia* L., is described and illustrated. Cultural features of single spore isolates are briefly noted.

Stagonospora macropycnidia sp.nov. (Figs. 1, 2)

Pycnidia dispersa, nigra, immersa, ellipsoidea, magna 410–1020 × 120–380 μ, papillata, poro 60–100 μ transverso. Sporulae hyalinae, cylindricae, rectae aut curvulae, utrinque obtusae, 3(5)-septatae, non constrictae ad septa, parvis olei guttis praesentibus, 25–70 × 2·5–5 μ.

Habitat in mortuis foliis Caricis pseudocyperus, circa Osterley, Middlesex, Anglia, 8 November 1952. Typus: Herb. I.M.I. 79901.

This fungus has been found several times between November and March on the dead leaf sheaths of *Carex pseudocyperus*, either submerged down to 30 cm. below the water surface or above water level but under saturated conditions. The pycnidia are usually on the sheaths of leaves formed at the bases of the inflorescence stems in the previous growing season. These stems arise from the middle regions of the densely tufted plants and, in consequence, their basal parts are always very wet. The dead sheaths are thin, papery and translucent when wet, and the pycnidia are easily seen.

It has also been found on submerged, dead leaves of *Carex acutiformis* in March and once on the thin edge of a leaf sheath of *Typha latifolia* in January. The *Typha* plant was in the middle of a large patch of *C. pseudocyperus* on which the fungus was also found.

The pycnidia are very large, appearing as conspicuous black spots on the thin 'host' tissue (Fig. 1 A). They are loosely gregarious and are often in lines owing to their position within the longitudinal air-spaces of the sheaths. They are immersed with the ostiole region projecting slightly above the leaf surface. Young pycnidia are spherical but the large mature ones are ellipsoid with their long axes parallel to the length of the leaf (Fig. 1 A, C). In transverse sections of the leaf the pycnidia appear to fill the air cavities (Fig. 1 B); they are prevented from enlarging uniformly during development by the longitudinal partitions in the leaf and thus become ellipsoidal as they expand along the air cavities.

The pycnidia are 410–1020 × 120–380 μ (av. of 50, 620 × 304 μ) with circular ostioles 60–100 μ diam. The wall is 20–35 μ thick and is composed of flattened, thin-walled cells, the outer layer of which is partially obscured by an encircling weft of brown hyphae, but in places they can be seen to be angular, 5–10 μ across, and to have colourless anticlinal walls (Fig. 1 D).

The colour of the wall when the pycnidium is crushed and viewed by transmitted light under low power is Tawny olive,* though round the pore it is darker.

The young spores are borne directly on the small colourless cells lining the inside of the pycnidial wall. They are very delicate, with thin walls and slightly granular contents and can be seen clearly only when stained with 0·01 % aqueous methylene blue (Fig. 2A). They become detached at an early stage and in a large but immature pycnidium many of them contain small oil drops, but they are not septate (Fig. 2B).

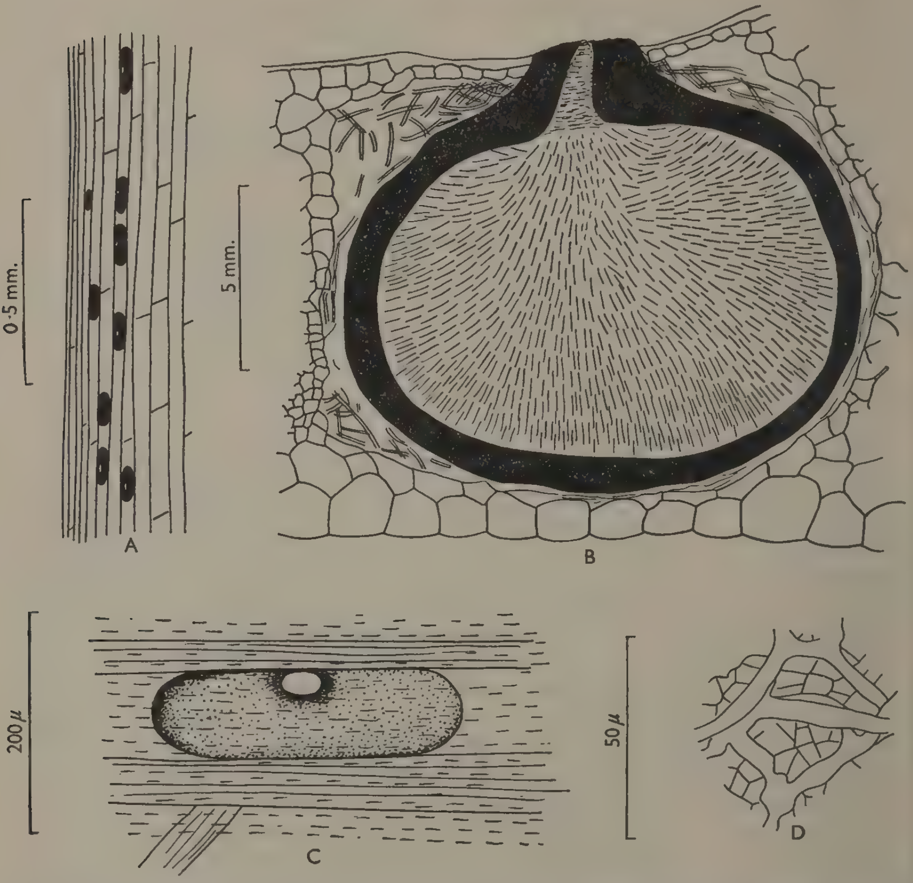

Fig. 1. *Stagonospora macropycnidia.* A, piece of leaf sheath of *Carex pseudocyperus* with pycnidia (× 5); B, vertical section of pycnidium (× 50); C, single pycnidium from above (× 150); D, part of outer wall of pycnidium (× 555).

The contents of a mature pycnidium consist of a white mass of many tightly packed spores which are oblong-linear, straight or sometimes curved and have obtuse ends (Fig. 2 C, D). There are usually three septa in each, though the longer spores may have five or more. The septa are very thin and difficult to see in the living spore, especially as the smooth lateral walls are not constricted. The position of a septum is often indicated by a group of small oil drops of uniform size on either side of it. Spore length depends upon the degree of maturity of the pycnidia from which the spore sample is taken.

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* Colour designations according to Ridgway (1912). *Color standards and color nomenclature.* Washington, D.C.

Although the spores are narrow in comparison with their length they are too wide for this fungus to be considered a species of *Septoria* and it seems most appropriate for it to be put in *Stagonospora*. About 109 species

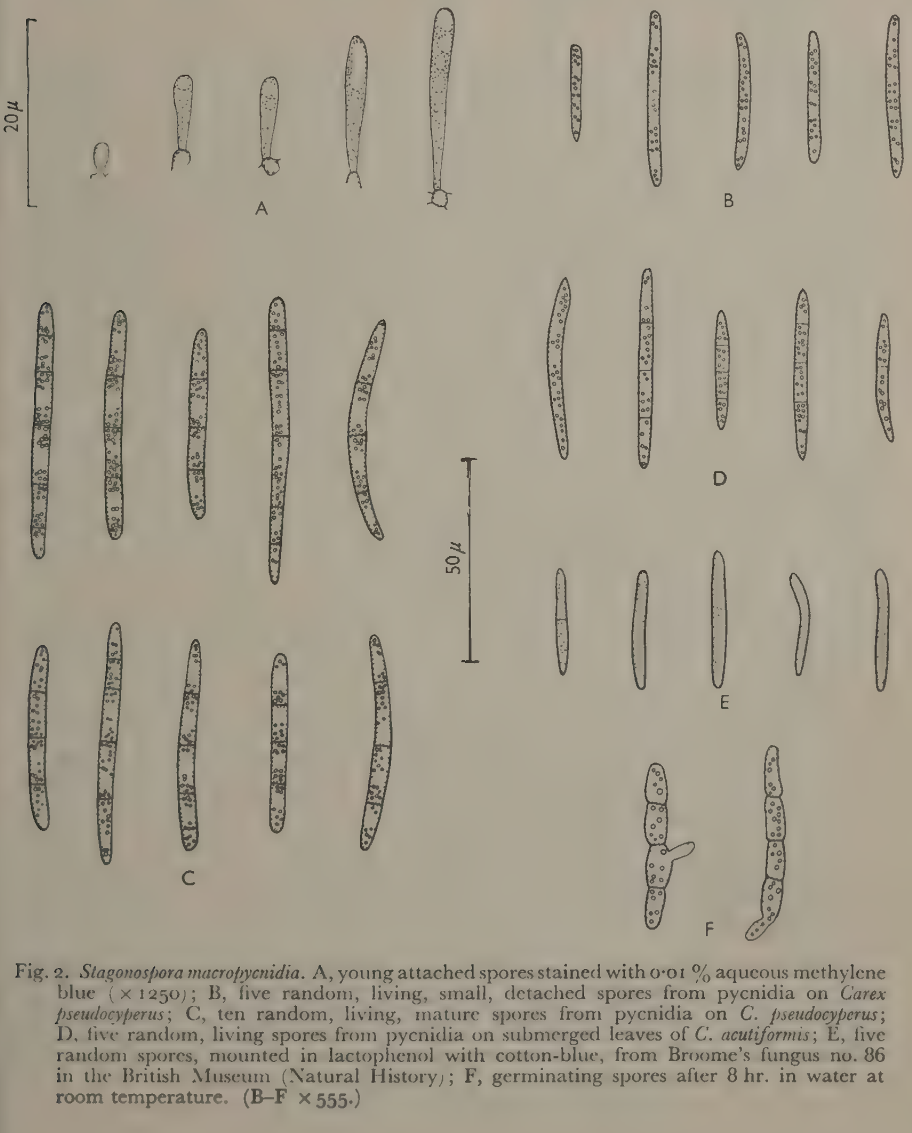

Fig. 2. *Stagonospora macropycnidia*. A, young attached spores stained with 0·01 % aqueous methylene blue (× 1250); B, five random, living, small, detached spores from pycnidia on *Carex pseudocyperus*; C, ten random, living, mature spores from pycnidia on *C. pseudocyperus*; D, five random, living spores from pycnidia on submerged leaves of *C. acutiformis*; E, five random spores, mounted in lactophenol with cotton-blue, from Broome's fungus no. 86 in the British Museum (Natural History); F, germinating spores after 8 hr. in water at room temperature. (B–F × 555.)

of *Stagonospora* have been described on monocotyledons, 24 on members of the Cyperaceae, 14 of these being on various carices. However, spore size and shape and also the extremely large pycnidia do not fit any of the published descriptions of these species. Therefore, it seems that it must be described as a new species, though fungi identical with it have almost certainly been found before. In the British Museum (Natural History) there is a specimen on a *Carex* leaf, labelled no. 86, which is on a sheet of unnamed *Hendersonia* spp. collected by C. E. Broome. The pycnidia are large and ellipsoidal and are very similar to those described above. The spores (Fig. 2E) are narrow, mostly straight and only rarely are any septa to be seen. They measure 22–42 × 2·5–4(5) μ (av. of 100, 27·5 × 3·4 μ), and agree perfectly with the detached immature ones of my material and undoubtedly belong to the same species. The correspondence between C. E. Broome and M. J. Berkeley concerning specimen no. 86 is also held by the B.M. (Natural History), but apparently these workers did not give it a specific epithet.

Dimensions of 100 *spores taken at random from three collections*

	Length (μ)			Width (μ)	
	Range	Mean	Standard deviation	Range	Mean
Carex pseudocyperus	40–70	50·8	7·06	2·5–4	3·4
C. acutiformis	25–56	40·1	6·75	3–5	4·0
Typha latifolia	41–60	50·0	3·54	3–5	3·9

There are two specimens in Herb. I.M.I. collected by M. B. Ellis from dead *Carex riparia* leaves which also appear to belong to this species (Wheatfen Broad, Norfolk, 25 May 1946, I.M.I. 34283; marshes near Flatford Mill, Essex, 7 May 1948, I.M.I. 28537). Although the spores are rather small (25 spores from the Flatford Mill material 20–42 × 2·5–4·5 μ, av. 33·6 × 3·2 μ) the pycnidia are typical.

The spores germinate readily in water at room temperature (Fig. 2F), and on 2 % malt agar a circular, non-staling colony is produced. At first the edge is evenly ragged but as the colony enlarges it becomes more irregular. The aerial mycelium is white, thick and cottony, and the reverse is also white. On potato dextrose agar and oatmeal agar growth is more rapid and the dense white mycelium often extends to the lid of the plate. The reverse is Cream Colour or Cartridge Buff. Old colonies on all three media retain the thick, white mycelium but the reverse becomes Cream Buff or Chamois and may be darker around the inoculum. On sterile *C. riparia* leaves a dense white cottony mycelium is formed. So far this fungus has not fruited in culture, though occasionally pale sclerotia, which may be incipient pycnidia, have been formed on 2 % malt agar and oatmeal agar.

I wish to thank the Keeper of Botany at the British Museum (Natural History) for permission to examine material and literature in his care. My thanks are also due to Dr M. B. Ellis for allowing me to examine his collections and to Mr B. C. Sutton for taxonomic advice.

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HELISCUS SUBMERSUS SP.NOV., AN AQUATIC HYPHOMYCETE FROM JAMAICA

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(With Plate 7 and 1 Text-figure)

Heliscus submersus sp.nov. is described from submerged decaying leaves and petioles. Its behaviour in culture is compared with that of *Nectria lugdunensis*.

Heliscus submersus sp.nov. (Pl. 7; Text-fig. 1)

Fungus aquaticus submersus, mycelio repente primo hyalino demum brunneo, septato, ramoso. Conidiophora hyalina brevia, 20–150 μ longa, simplicia vel ad apices ramosa, quoque ramo 1–4 phialides gerentia. Conidia (phialosporae) acrogena, hyalina, recta vel curvula, continua vel 1-septata, 29–51 μ longa, 1·5–2·5 μ lata ad basem, apicem versus ad 3–4·5 μ, 2–3·5 μ lata ad septum, projecturis conicis duobus ad apicem 4–8·5 μ longa et 2·5–3·5 μ lata.

Habitat in foliis putrescentibus in flumine jacentibus Hardwar Gap, St Andrew, Jamaica. Typus Herb. I.M.I. 76792.

In a survey of Jamaican aquatic hyphomycetes Hudson & Ingold (1960) briefly mentioned a species of *Heliscus* collected on decaying leaves, especially the petioles, from a stream at Hardwar Gap, St Andrew, and the River Negro at Cedar Valley, St Thomas. At first it was thought that this might be a form of *Heliscus lugdunensis*, the conidial state of *Nectria lugdunensis* Webster (1959), but further collections and a study of the fungus in pure culture have shown that this differs sufficiently to be described as a new species.

Collections of leaves and petioles kept in dishes of aerated water in the laboratory produced within 24 hr. crops of conidiophores, either simple or sparingly branched and up to 150 μ long and 9 μ wide. Each branch produces 1–4 phialides, usually 3, measuring 9–12 × 3–4 μ. Each mature phialospore is hyaline, T or Y-shaped and straight or slightly curved (Text-fig. 1 A, B; Pl. 7 A, B). In the young club-shaped phialospore the growth of the original apex is arrested and a new growing point is initiated which grows out laterally and opposite to the original one, giving the spore it characteristic shape. The mature phialospores have an overall length of 29–51 μ and are 26–44 μ from their attachment to the phialide to the point of divergence of the arms. They are 1·5–2 μ wide at the base, 3–4 μ wide below the arms and 2–3·5 μ wide at the single septum, if present. This septum is usually formed immediately before, or just after the spore is liberated and occurs 20·5–24 μ from the base. The two arms are 4–8·5 μ long and 1–2 μ wide at the tip, diverging to 2·5–3·5 μ at the body of the spore.

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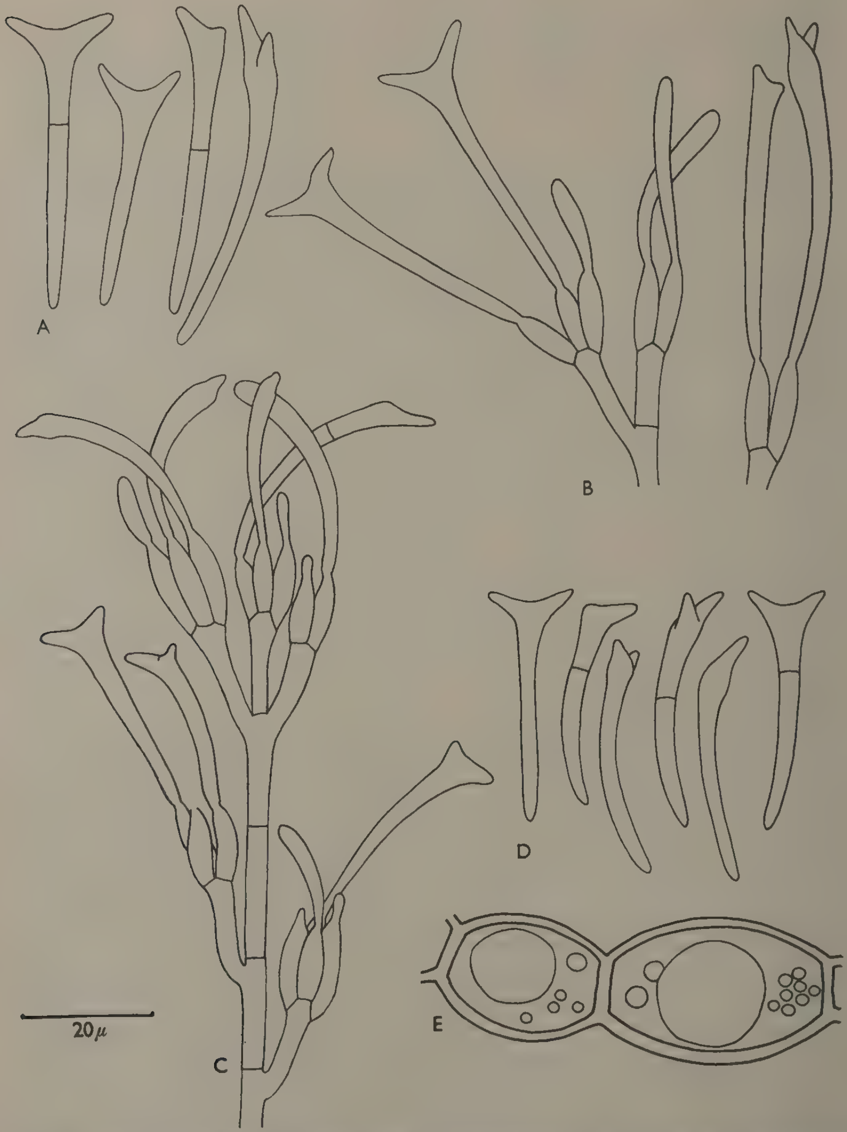
* Now at The Botany School, Cambridge.

Single spores were isolated and these germinated rapidly, usually from the three extremities. On 2% malt extract agar the fungus forms at first a white slow-growing colony with sparse aerial mycelium. The colony soon turns brown by producing a light brown aerial mycelium with a much darker pigmentation near the surface of the medium. This darker coloration



Text-fig. 1. *Heliscus submersus.* A, Phialospores; B, phialospores in various stages of development; both from leaves in a stream at Hardwar Gap; C, conidiophore and phialospores from a submerged culture; D, mature phialospores formed aerially on 2% malt agar; E, chlamydospores from culture.

often occurs in concentric rings with lighter brown areas in between. With age a fluffy aerial mycelium is formed which shows a tendency to aggregate into rope like strands of ten or more hyphae. Phialospores are produced abundantly in culture at laboratory temperatures and differ in no way from those produced even more profusely when slices from a colony are placed in sterile aerated water (Text-fig. 1 C, D; Pl. 7 D, F). The aerial conidiophores, which are simple or occasionally branched, are up to 140 μ long and produce sticky heads of phialospores 20–95 μ diam. (Pl. 7 E). Phialospore clusters are also borne on the surface of the medium and buried within it. Phialides may be produced on horizontal hyphae and this is also true in submerged aerated cultures.

A further feature of the fungus in culture is the production of irregularly branched chains of chlamydospores on the aerial mycelium where they appear as dark brown knots. The chlamydospores are spherical to elongate, measuring 24–40 × 15–28 μ. They have a dark brown pigmented wall, 1–2·5 μ thick, and each usually possesses a single large oil guttule together with several smaller ones (Text-fig. 1 E and Pl. 7 C). As the culture ages, small sclerotium-like bodies, 160–460 μ diam., are formed. These are dark brown to black, formed within the media and are more abundant towards the margin of the colony.

Of the four species of *Heliscus* described, *H. submersus* most resembles *H.* (*Nectria*) *lugdunensis* (syn. *H. aquaticus* Ingold). It differs markedly in spore form from *H. tentaculus* Umphlett, *H. longibrachiatus* Ingold and *H. stellatus* Ingold & Cox and in culture from the last two species. *H. lugdunensis* Sacc. & Therr produces cylindrical-clavate to clove-shaped phialospores, up to 3-septate and 32–42 × 7–8 μ, whereas the phialospores of *H. submersus* are never more than 2-celled and are 29–51 × 3–4 μ. They form only two conical arms at the apex, in contrast to *H. lugdunensis* which normally produces three arms and also microconidia, which have not been observed in *H. submersus*.

In culture they resemble each other more than any one of the other species, in that they produce a fluffy aerial mycelium, pale brown in the centre with white margin, and they both show a tendency for the aerial mycelium to aggregate into strands. But they differ markedly in growth rates (Table 1).

Table 1. *Comparison of* Heliscus submersus *and* Nectria lugdunensis *in culture*

		Colony diam. (mm.) at 20° C. (average of 5 replicates)		
		7 days	10 days	14 days
H. submersus	2 % malt extract agar	21	30	36
	Potato dextrose agar	20	31	37
N. lugdunensis	2 % malt extract agar	28	42	55
	Potato dextrose agar	33	50	67

Chlamydospores have not been described for *N. lugdunensis* and Webster (1959) does not mention sclerotium-like bodies. Ingold (1942), in his description of the species under *H. aquaticus*, described irregular, small, dark brown sclerotium-like bodies, 0·3–1·0 mm. diam., found below the surface of the medium. The phialospores of *H. submersus* show a closer resemblance to those of *H. aquaticus* (Ingold, 1942, Text-fig. 14A) than those of *N. lugdunensis* (Webster, 1959, Text-fig. 1E). But in *H. submersus* there are never three diverging arms and the phialospores borne aerially are identical with those produced in water.

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EXPLANATION OF PLATE 7

A, mature phialospore from stream water (× 1000).
B, two developing phialospores and phialides from a leaf (× 1000).
C, chlamydospores from culture (× 250).
D, mature phialospores produced from culture in aerated water (× 1000).
E, conidiophore with sticky head of conidia from a dry culture (× 200).
F, conidiophore produced in submerged culture (× 500).

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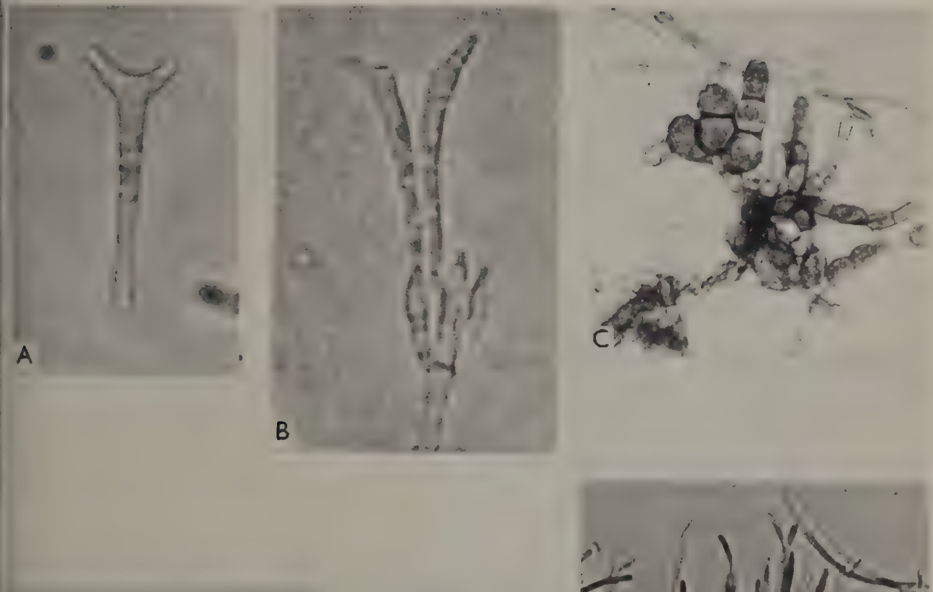
A
B
C
D
E
F

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FRUIT-SPOT ('SPECKLE') OF JAMAICAN BANANAS CAUSED BY *DEIGHTONIELLA TORULOSA* (SYD.) ELLIS

I. SYMPTOMS OF DISEASE AND STUDIES ON PATHOGENICITY

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(With Plates 8 and 9)

The external symptoms of a serious fruit-spot ('speckle') of Jamaican 'Lacatan' bananas are described. Spots are round, up to 4 mm. in diam., with a reddish brown centre and a characteristic dark green halo. *Deightoniella torulosa* was isolated with a high frequency from spotted tissue; no other fungus (or bacterium) was isolated with significant frequency. In pathogenicity tests, *D. torulosa* caused typical spots to develop on the fruit peel after 48 hr. After 72 hr. some spots had diameters of approximately 3 mm.

Conidia of *D. torulosa* germinate after 1 hr. in a film of water on the surface of banana fruit peel. One or two appressoria are produced by each conidium. The appressorium develops a fine infection hypha which penetrates the cuticle and epidermal wall by mechanical means. This stage in infection was observed after 12–20 hr. Further tissue invasion occurs by hyphal penetration of cell walls. Shortly after infection, cell walls and contents develop a reddish brown colour and may contain large numbers of spherical inclusion-bodies. Similar stages in this infection process were observed on naturally infected fruit. These results are discussed in relation to 'Johnston fruit-spot', which occurs in Central and South America.

Deightoniella torulosa (Syd.) Ellis was first recorded as *Brachysporium torulosum* Syd. on dead leaves in Brazil (Sydow & Sydow, 1909—cited by Wardlaw, 1935). Ashby (1913) recorded the same fungus as *Cercospora musarum* Ashby, causing a leaf-spot of 'Gros Michel' bananas in Jamaica. In 1928, the fungus was renamed *Helminthosporium torulosum* (Syd.) Ashby (Mason, 1928) and only recently was it included in the genus *Deightoniella* (Ellis, 1957).

D. torulosa is very widely distributed (Commonwealth Mycological Institute, Distribution Map No. 175) and has been recorded on leaves and fruits in most banana-growing regions. Mason (1928) observed the fungus on banana suckers collected in West Africa by R. H. Bunting. The fungus has also been isolated from rotten stem tissue of the Manila hemp plant (*Musa textilis*) in the Philippines (Calinisan, Agati & Aldaba, 1931), Malaya (Anon., 1952) and New Guinea and Papua (Thrower, 1954).

With few exceptions, previous references to *D. torulosa* on bananas have concerned a leaf disease ('black-spot', Ashby, 1913; Wardlaw, 1934*b*; Stahel, 1934), or a fruit-tip discoloration ('black-tip', Ogilvie, 1928; Wardlaw, 1932). Wardlaw (1934*a*) studied fruit-spots occurring on 'Gros Michel' and 'Cavendish' varieties in Trinidad, but the typical fruit-spot occurring on Jamaican 'Lacatan' bananas was not described, presumably because it was not abundant. From spotted tissues, Wardlaw isolated several different fungi, of which the most common were *D. torulosa*, *Piricularia grisea*, *Gloeosporium musarum*, *Pestalozzia leprogena*, *Phomopsis* sp. and *Fusarium* spp. Since no pathogenicity tests were conducted, it was impossible to decide whether these fungi were acting in a primary or secondary capacity.

Johnston (1932) and Johnston & Slocum (1932) have described a spotting of 'Gros Michel' bananas in several Central and South American countries. These workers were unable to find any evidence for insect or fungal invasion (bacteria and viruses were not considered), and concluded that spotting was a physiological disorder, due to such factors as heavy rainfall and high atmospheric humidity, and a low nitrate content of the soil. Of the types of spots recorded in the literature, the 'Johnston fruit-spot' is most like the Jamaican form.

A few other references to banana fruit-spots may be noted. A 'pitting' disease of the finger-stalks and cushions was first described by Tomkins (1931) and was subsequently investigated by Wardlaw & McGuire (1931, 1932) and by Wardlaw (1934*a*). Although *D. torulosa*, *G. musarum* and other fungi were occasionally isolated, the high frequency with which *Piricularia grisea* appeared on isolation plates led Wardlaw to suggest this as the causal organism. In Italian Somaliland, Petri (1932) found *D. torulosa* associated with black pits on banana fruits: however, the same type of pitted tissue often yielded *G. musarum*, *Stachylidium* (*Verticillium*) *theobromae* and *Fusarium* sp.

It is thus evident that *D. torulosa*, although obviously associated with several types of fruit-spot, has in no instance been proved to be the causal organism. The present paper describes some preliminary observations which suggest that this fungus is responsible for at least one form of fruit-spot.

FRUIT-SPOT IN JAMAICA

Economic importance

In Jamaica, the fruit-spot described here is known as 'swamp-spot' or 'speckle'. Leach (1959) considers that 'speckle is one of the most disconcerting of all banana diseases in Jamaica due to its unknown cause and because at times the fruit can be very seriously disfigured by it'. Speckle affects only the outer peel tissues of the banana and, therefore, even when the disease is severe there is no wastage due to inedibility. But the unattractive appearance of badly speckled fruit usually results in its being rejected for marketing. It is difficult to obtain reliable data concerning the wastage due to speckle, for trade reports merely provide an overall estimate for wastage caused by mechanical injury, fruit-rot, speckle, immature and over-mature fruit, etc. Nevertheless, during the autumn and early spring months, corresponding with the periods of heaviest rainfall in Jamaica, speckle would appear to be one of the most serious sources of wastage.

External symptoms of disease

Speckling may be observed in the field on immature fruit. It is sometimes apparent a few days after the bunch has 'shot' (i.e. emerged from the top of the pseudostem). In these cases the individual spots are usually few in number and widely scattered over the fruit surface. The severity of speckling increases with maturity of fruit and disease is most noticeable at the time of harvest, especially after periods of prolonged and heavy rainfall.

On green fruit, disease is first manifest as minute, reddish brown spots just visible to the naked eye. A hand-lens will show that each spot is surrounded by a halo of dark green, water-soaked tissue. The centre of the spot may retain the reddish brown colour but sometimes it becomes black; occasionally it becomes fawn coloured, thus presenting a corky appearance. As the spot increases in diameter, the halo becomes more distinct and usually has a diameter two or three times greater than that of the dark centre. There is considerable variation in the diameter of spots on a given finger; some are just visible while the largest have diameters up to 4 mm. including the water-soaked halo. Spotting may be so dense that the halos of adjacent spots overlap (Pl. 8, fig. 1). The centre of the spot is depressed slightly and may become cracked as the fruit becomes 'fuller.'

There is a characteristic distribution of spots on the bunch, the large proximal hand (known as the 'butt-end' hand) being more severely affected than the smaller, more distal hands. The distribution of spots on each finger is also characteristic: on the outer face, they are most abundant towards the tip (blossom-end) (Pl. 8, fig. 1), while on the inner face the tend to be concentrated towards the finger-stalk (stem-end) and even on the cushion. The outer faces of the inner whorl of fingers on any given hand are commonly less spotted than those of the outer whorl. Conversely, the inner faces of the inner whorl tend to be more spotted than those of the outer whorl. In general, spots on the outer faces are larger than those on the inside of the bunch. It often happens that there is much spotting of the inner faces of fingers in the inner whorl but little or none on the outer faces of either whorl. Thus it is possible mistakenly to record a bunch viewed from the outside as being unaffected, or only lightly so.

Some observations were made on immature bunches still attached to wind-blown plants. Within a few days of being blown down, the fingers on the uppermost side of the bunch became very severely spotted, while those on the underside were almost free from spotting. This intense spotting was particularly evident when there had been rain showers after blowing down. In contrast, bunches on upright neighbouring plants showed little or no increase in density of spotting. A few plants bearing immature fruit were pulled down intentionally and the fruit kept under constant observation. After 2–3 days, there was a great increase in the number of spots on the upper side of the fallen bunch but no appreciable increase on the underside. In this case also there had been rain showers during the 2- to 3-day period.

Further observations on fruit-spot were made during cool storage and ripening. Severity of spotting appears to increase during transport from Jamaica to the United Kingdom. At certain times of the year, many severely spotted stems are rejected immediately after discharge from the ship's hold. It is unlikely that all of this fruit was severely diseased at the time of loading, otherwise it would not have been passed as fit for export. In the current investigation, some lightly spotted fruit was harvested at the 'light-full three-quarters' stage of maturity and transferred immediately to a cool chamber maintained at 55° F. After 12 days storage, the severity of spotting was assessed. Some of the spots present at harvest had increased slightly and some new spots had developed.

The development of spots was also followed throughout the ripening process. Fruit was stored for 7 days at 68° F., by which time it was fully ripe. As the peel colour changed from green to yellow ('turning'), the dark green halo around each spot became less distinct so that, on the ripe fruit, only the original dark centre of the spot was visible. There was no appreciable increase in diameter of the dark region during ripening.

Histological studies on spotted tissue

Since it is difficult to strip off the epidermis of banana fruits, surface hand sections, two to four cells thick, were prepared. In a typical spot, the central epidermal cells are filled with a reddish brown or black substance resembling gum or tannin; cell walls are similarly stained. Each cell usually contains one or several brown, spherical inclusion bodies which may be minute or nearly fill the cell lumen (Pl. 9, fig. 2). Epidermal cells at the periphery of the dark area contain less of the gummy or tannin-like substance, and the inclusions are of a lighter colour. In the region of the halo, there is little darkening of cell contents and walls and the inclusions, although numerous, are almost hyaline. Towards the periphery of the halo the number of cells containing inclusions decreases. These characteristic inclusions cannot be distinguished in lactophenol-mounted sections, apparently due to their being 'dissolved'. In surface sections it is common to observe single epidermal cells and small groups of cells with contents that are reddish brown or black and which contain the characteristic spherical inclusions; these cells may represent early stages of spot development. There is no constant relationship between the position of spots and that of stomata.

In vertical sections cut 8–10μ thick with a microtome, the outline of each spot is nearly semicircular. At the centre the epidermis is very slightly depressed, and the cells, and a core of peel parenchyma cells, are usually darker than surrounding ones. The depth to which necrosis extends into the peel appears to be related to the external diameter of the spot. In the largest spots (4 mm. diameter), twelve to twenty cell layers were affected; at the other extreme, only one epidermal cell might be necrosed, as noted above. There is some shrinkage of discoloured cells and the cell outline may be more irregular than that of apparently healthy cells. In his studies on fruit-spot, Johnston (1932) 'consistently observed that a vascular strand, and internal to it a latex tube, lay immediately below each spot'. This was not always the case in the present study.

Isolation of the pathogen

Preliminary tests designed to ascertain whether fruit-spot is caused by bacterial infection, using the techniques described by Dowson (1957), gave negative results. Since the surface of the banana fruit bears large numbers of fungi, yeast and bacteria, attempts to isolate fungi from diseased tissues were made only after rigorous surface sterilization. Immature spotted fruit was surface sterilized by immersion for 5–10 min. in 0·1 % mercuric chloride, rinsing in 50 % alcohol and then flaming. Some of the smallest spots were cut out with a previously flamed knife and transferred to the surface of potato-dextrose agar. In many cases mycelium grew out of spotted tissue after 2 days incubation at 28–30° C. After further incubation for 6–7 days, the cultures were examined; in every case, the fungus isolated was *D. torulosa*. From one isolation series, thirty-six out of forty spots yielded this fungus; four plates remained sterile, possibly due to the use of a hot knife and too severe surface sterilization of fruit. *D. torulosa* was isolated from large and small spots and, so far, only in one instance has another fungus been isolated (*Gloeosporium musarum*). An account of the cultural characters of *D. torulosa* is given by Wardlaw (1932); the characteristics of his isolates agree closely with those of the isolates obtained in the present investigation.

EXPERIMENTAL INFECTION BY *DEIGHTONIELLA TORULOSA*

Pathogenicity tests

An abundant supply of conidia of *D. torulosa* was produced on cultures on a starch medium prepared as described by Wardlaw (1932).* Abundant sporulation was also obtained by culturing on sterilized banana leaf (Stahel, 1934). Preliminary tests were conducted in the laboratory. Immature ('thin') bananas were surface sterilized and pieces of completely spot-free peel, measuring approximately 3·0 × 2·0 × 0·5 cm., were cut out. To ensure that no spotted peel was used, each piece was examined under a low-power dissecting microscope before being placed in a sterile Petri dish containing moist filter-paper, and inoculated with one drop of a water suspension of conidia of *D. torulosa* from a pure culture. Control pieces of peel were treated with sterile water only. After 30 hr. incubation at 28° C., many minute reddish brown spots, each having a characteristic dark green halo, appeared on all pieces of inoculated peel (thirty-five replicates). In no instance did spots develop on controls. After 72 hr., the diameter of some spots had increased to nearly 1 mm. Detailed macroscopic and microscopic examination of these spots showed that they were identical with those occurring on naturally infected fruit. After further incubation, more spots appeared, while those that were already present continued to increase in diameter. At the conclusion of the experiment (90 hr.), by which time most of the peel segments were rotten or badly discoloured, the maximum diameter recorded was approximately 2 mm. *D. torulosa* was consistently re-isolated from surface-sterilized, spotted tissue; no other species was isolated. The experiment was repeated several times and in each case the same results were obtained, thus providing strong evidence that *D. torulosa* is the causal organism of fruit-spot.

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* Wardlaw's medium is prepared as follows: to 1000 ml. distilled water add 1·25 g. potassium phosphate, 0·75 g. magnesium sulphate, 2·0 g. asparagin, 25 g. starch and 20 g. agar. Boil, filter and autoclave for 20 min. at 15 lb. pressure.

Further inoculations were carried out in the plantation. Immature bananas (approximately 40 days from the time of 'shooting') were surface sterilized by wiping the skin with spirit-soaked cotton wool. After evaporation of the spirit, a dense suspension of conidia of *D. torulosa* (from pure culture) was sprayed onto each finger until run-off occurred. Control fruit was sprayed with sterile water only. Each inoculated finger was then covered by a plastic bag, firmly tied at its open end in order to maintain a high humidity near the fruit epidermis. After 2 days, minute characteristic spots developed on fruit inoculated with *D. torulosa*; control fruit was not spotted. After 5 days the spots were quite large (up to 0·5 mm. in diameter). Thus the results of laboratory tests were confirmed.

Germination of conidia on surface of host

Germination at 28° C. was observed in water films on the surface of apparently healthy immature banana fruits. After 1 hr., most conidia observed had produced a very short germ tube at one or both ends. After 3 hr., some germ tubes had attained a length of approximately 80μ; the mean germ-tube length was $40–50\mu$. In a few instances, the germ tube had extended for less than 20μ before becoming septate and swelling at the tip into a hyaline, round appressorium. The majority of conidia developed appressoria after 6 hr. There was no tendency for germ tubes to grow towards stomata and only rarely did an appressorium develop above a guard cell. At 6 hr., some appressoria developed an irregular, lobed outline and contained a bright circular area; it is probable that this area indicated the position of the infection hypha which had not yet penetrated the cuticle. The appressorium gradually developed dark-coloured granular contents so that its outline was readily observed in surface sections of peel tissue. After germination, the endoconidial septa were slowly dissolved and the conidium wall, consisting of a thin exosporium and thick endosporium, collapsed. Identification of collapsed, germinated conida was facilitated by the persistence of the characteristic black annulus at the base, where attachment was made to the swollen conidiophore head. From these observations and from those of Stahel (1934) germination of *D. torulosa* conidia on fruit and leaves appeared to follow a similar course.

Germination was also observed in water films on a glass surface. A notable feature was the considerable length to which some germ tubes developed ($> 500\mu$) before the production of appressoria. Thus appressorium development was considerably delayed relative to that on fruit. Another difference was the extensive branching of germ tubes on glass, this being of rare occurrence on the epidermis of the fruit.

Penetration of the host and pathological histology

Details of penetration were studied on the pieces of peel tissue that were used for germination tests, by means of surface sections, one or two cells thick, stained with cotton blue in lactophenol. After 12–20 hr., the wall and contents of the epidermal cell underlying the appressorium became reddish brown, indicating that penetration had occurred. In many sections, the swollen infection hypha was seen inside the epidermal cell, and by further focusing it was found that the hypha had penetrated into an underlying hypodermal cell (Pl. 9, figs. 3, 4). By 24 hr., groups of fifteen or more adjacent epidermal cells were similarly discoloured. These lesions could be detected on the fruit surface through a hand-lens, and the characteristic spherical bodies appeared inside dead cells. It was not infrequent for two or more appressoria to be associated with a spot. These were probably instances of multiple infection.

Penetration was also observed in vertical microtome sections, 8–10μ thick, stained with cotton blue in lactophenol; these showed the appressorium pressed closely against the surface of the cuticle, usually in the grooves between epidermal cells. Shortly before the infection hypha is produced, the epidermal wall becomes dented, probably due to mechanical pressure from the appressorium. The infection hypha is a relatively fine structure but, after penetrating the cuticle and epidermal wall, it swells appreciably. The next cell to be invaded is usually hypodermal, the infection hypha penetrating the cell walls (Pl. 9, fig. 5).

The fungus traverses the skin tissues by cell to cell penetration. No hyphae were observed in the middle lamellae or intercellular spaces. When a hypha approaches a cell wall it swells (Pl. 9, fig. 6) and then produces a fine hypha which effects penetration. Once through the cell wall, the hypha swells once more. Branching of hyphae occurs in many cells.

NATURAL INFECTION BY *DEIGHTONIELLA TORULOSA*

Observations were designed to trace the course of infection by *D. torulosa* under natural conditions. The presence of large numbers of germinated and ungerminated conidia of *D. torulosa* on the surface of heavily spotted fruit was demonstrated repeatedly, both by taking washings and by direct microscopic examination of surface sections. On such fruit, all stages of infection were evident. The earliest indication of disease was the presence of a single epidermal cell having a reddish brown wall and similarly coloured, granular contents. With few exceptions, an appressorium (occasionally two) was observed lying on the surface of the cuticle immediately above the dead cell. Most of these appressoria were still attached to the germinated conidium (Pl. 9, fig. 7). In contrast to their behaviour on experimentally inoculated fruit, naturally occurring germ tubes are relatively short (10–50μ). Possibly this is an adaptation to natural environmental conditions, which may be less favourable for the fungus than experimental ones.

By carefully focusing with an oil-immersion objective, it was sometimes possible to detect the infection hypha in the first-entered cell. Although many vertical sections of naturally spotted fruit have been prepared, so far it has not been possible to observe the actual process of penetration.

Earlier stages in the infection process were probably represented by appressoria that were lying on the surface of apparently healthy epidermal cells. In these appressoria there was often a bright circular area, possibly corresponding with the position of the infection hypha that had not yet penetrated the cuticle.

Many larger spots were examined in surface sections. Those in which two to twelve epidermal cells were necrosed did not usually require clearing in order to see the outlines of individual cells. In nearly every instance it was possible to detect the collapsed remains of the appressorium (sometimes more than one) lying on the cuticle. Often, the remains of the conidium were still attached by means of the germ tube. Spots with diameters exceeding about 1 mm. were first cleared in lactophenol: even here the remains of conidia and appressoria were frequently observed. Detection of these structures was facilitated by mounting sections in 1 % cotton blue in lactophenol and examining with the aid of a green light-filter. Vertical sections stained in lactophenol showed that the distribution of hyphae in diseased tissues was similar to that in experimentally inoculated fruit.

Inoculum sources

D. torulosa grows as a saprophyte on dead and dying banana leaf laminae and petioles, on which it also sporulates abundantly at certain times. Factors affecting sporulation on this material have not yet been investigated. This distribution of inoculum suggests that good plantation hygiene, e.g. removing dead 'trash' leaves, may have some effect in reducing the incidence of fruit-spot. There is no evidence of *D. torulosa* sporulating on the surface of the fruit-spot.

Discussion

The evidence obtained from pathogenicity tests, observations on naturally occurring infection and histological studies justifies the conclusion that fruit-spot ('speckle') of Jamaican 'Lacatan' bananas is caused by *D. torulosa*. Details of infection of fruit are similar to those described by Stahel (1934) for leaf infection. The black lesions produced on leaves are very much larger than typical fruit-spots. It is possible that physiological conditions in fruit tissue are not so favourable for the fungus as are those in leaves. Even when the fungus is inoculated into wounded bananas, the extent of internal rotting is insignificant, infection being mainly confined to the superficial tissues (Wardlaw, 1932).

Of the fruit-spots mentioned in earlier literature, those described by Johnston (1932) and Johnston & Slocum (1932) bear the closest resemblance to 'speckle'. Until suitable material becomes available for comparison, it is not possible to determine whether they are, in fact, identical. If the 'Johnston fruit-spot' is caused by *D. torulosa*, several of these workers' observations could be explained tentatively. Thus they found that the season of most severe spotting coincides with the season of heaviest rainfall, high humidity and low evaporation: a similar coincidence is evident in the case of Jamaican fruit-spot. The negative evidence from mycological and entomological studies led Johnston (1932) and Johnston & Slocum (1932) to consider spotting as a physiological disorder. However, these workers were often able to detect hyphae in old spots but not, apparently, in small ones—a finding that requires confirmation.

In Central and South American banana plantations, some reduction in fruit-spot has been effected by covering the bunch with a plastic bag shortly after 'shooting' (United Fruit Company, Boston, 1959). The presence of bags probably reduces the amount of inoculum of *D. torulosa* that is able to impact on the fruit surface.

It was noted that wind-blown fruit often develop severe spotting after a short period on the ground, particularly during rainy periods. The chief sources of conidia of *D. torulosa* in plantations are the dead or dying leaves that hang down from the pseudostem. The downward movement of large numbers of conidia in rain showers probably accounts for the high incidence of spotting on wind-blown fruit. Because in the 'Lacatan' variety the fruit usually hangs at a level above the collapsed leaves, it seems likely that there is less contamination by water-borne conidia in this variety of banana.

From his descriptions it is unlikely that many of the spots investigated by Wardlaw (1934*a*) are identical with 'speckle'. However, it is interesting to note that in one of Wardlaw's photographs (1932, Fig. 8), in which 'black-tip' of 'Lacatan' fruit is illustrated, several typical 'speckles' are evident—an example of two different diseases caused by the same fungus, *D. torulosa*, occurring on the same banana.

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EXPLANATION OF PLATES 8 AND 9

Plate 8

Fig. 1. Recently harvested bunch of 'Lacatan' bananas severely affected by 'fruit-spot' ('speckle', 'swamp-spot'); in many spots it is possible to distinguish the dark centre surrounded by a lighter coloured halo.

Plate 9

Fig. 2. Tangential section of fruit-spot showing necrosis and inclusion-bodies in epidermal cells.

Fig. 3. Germinated conidium of *D. torulosa* with germ tube and swollen appressorium. One epidermal cell is already infected and has become darker than adjacent, healthy cells.

Fig. 4. A hypodermal cell immediately underlying the infected epidermal cell shown in Fig. 3; hyphae of *D. torulosa* are visible in the cell lumen.

Fig. 5. Germ tube and inverted funnel-shaped appressorium of *D. torulosa* attached to banana fruit epidermis; in one hypodermal cell, a swollen hypha can be seen.

Fig. 6. Intracellular hyphae of *D. torulosa* in fruit hypodermis.

Fig. 7. Naturally occurring germinated conidium of *D. torulosa* on the surface of banana fruit epidermis; a short germ tube was produced before the lobed appressorium was formed. Identification of the conidium is facilitated by the persistent black annulus at the base through which attachment to the conidiophore was originally made. A few cells underlying the appressorium are already infected, as evidenced by their darker contents. A similar illustration of a germinated conidium on banana leaf epidermis is given by Stahel (1934), opposite p. 142 of his paper.

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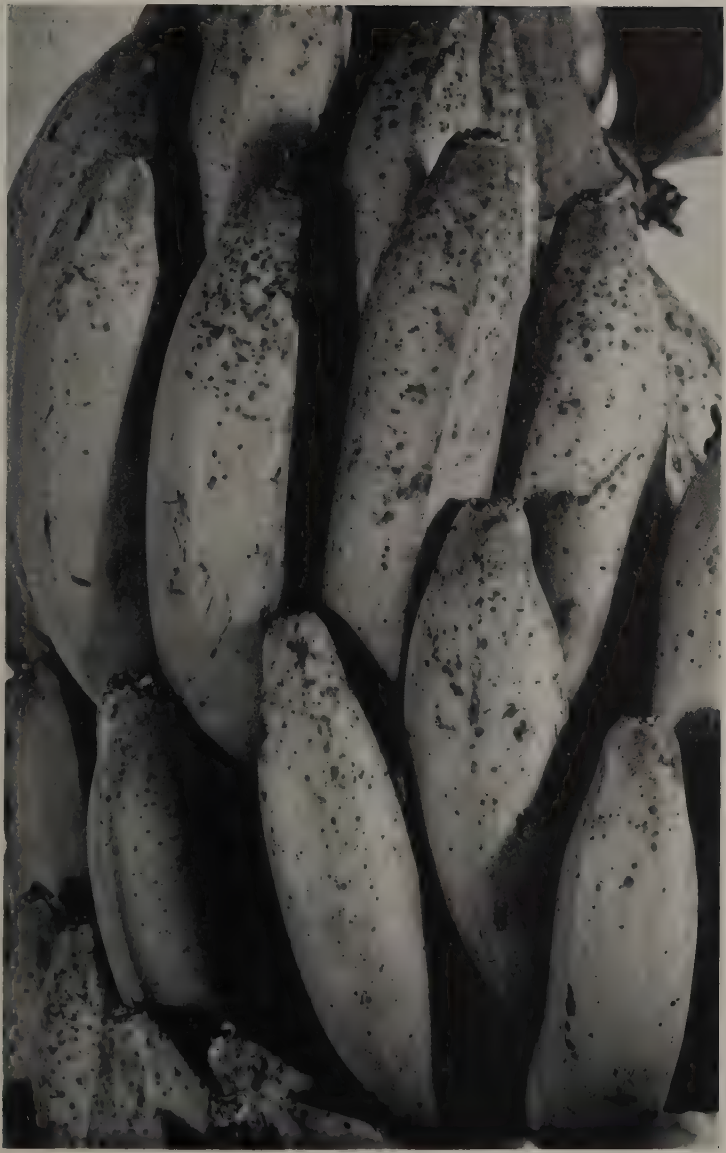

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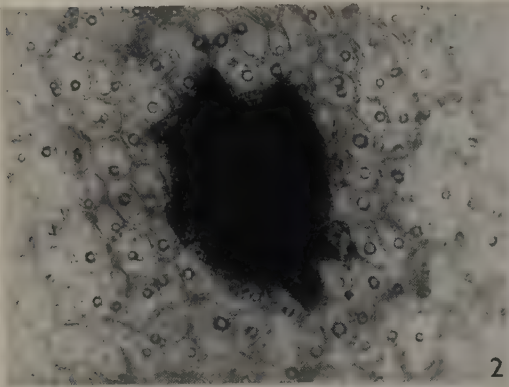

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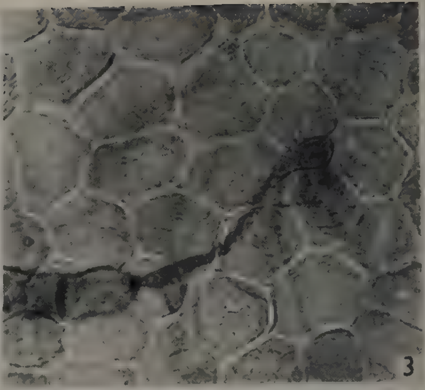

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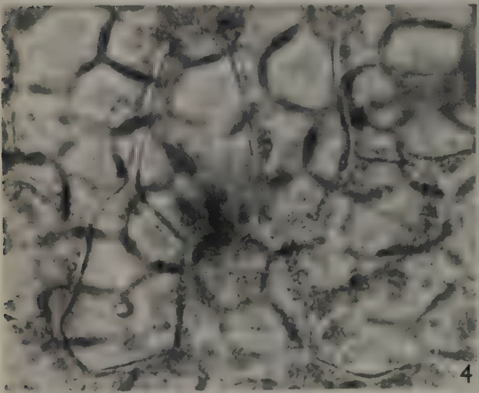

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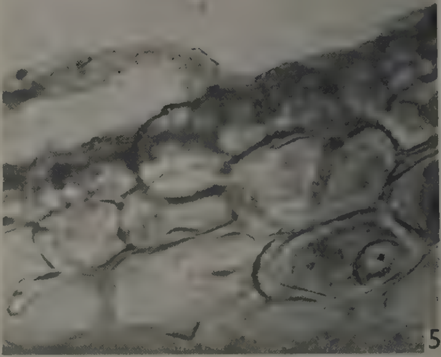

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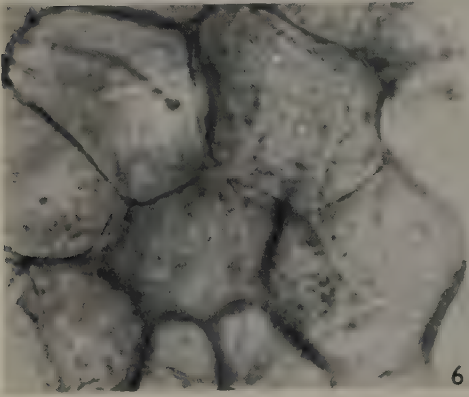

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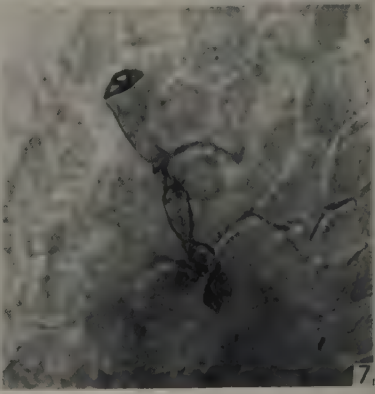

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Trans. Brit. mycol. Soc. **44** (1), 105–118 (1961).

SAPROPHYTIC ACTIVITY OF *PYTHIUM MAMILLATUM* IN SOILS

II. FACTORS RESTRICTING *P. MAMILLATUM* TO PIONEER COLONIZATION OF SUBSTRATES

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Pythium mamillatum Meurs did not colonize organic material containing a well-established flora of sugar fungi, cellulose-decomposing, or lignin-decomposing fungi. This fungus is thus limited to the pioneer position in an ecological succession. Experiments designed to elucidate the antagonistic mechanism operative here showed that specific antibiotics or competition for nutrients or space were not necessarily involved. The antagonism was effected through the agency of general staling substances, which were dependent on a high density of parent hyphae for production in sufficiently toxic concentration. This conclusion is discussed in relation to the biology of *P. mamillatum* in soils, and to the wider problem of antagonisms in this habitat.

Valuable information concerning the ecological behaviour of certain higher plants has been gained by subjecting them to the environmental conditions of an abnormal ecological niche (Turesson, 1922; Clausen, 1951). Since ecology is essentially a single science (Garrett, 1952), it is not surprising to find that tenets deduced from higher-plant ecology can often be effectively applied to the microbial ecology of soils. The results of a basically similar series of experiments with *Pythium mamillatum* in soil, therefore, are presented herein. The ability of this fungus to colonize, from soil, an organic substrate already containing an established flora has been investigated; this experimental procedure places the fungus in the position of a possible colonizer at a much later stage in the succession than is usual for it. The results obtained, whilst essentially contributing towards a more complete understanding of the biology of *P. mamillatum* as a sugar fungus in soil, nevertheless touch broader problems of antagonism in soil, and are finally discussed in relation to these two problems.

MATERIALS AND METHODS

The materials and methods employed in the present work have been described fully elsewhere (Barton, 1958, 1960*a*, *b*). Very briefly, two soils were used throughout; soil A was a Lancashire garden soil (pH 5·4) from which *P. mamillatum* was originally isolated, and soil B was a woodland soil (pH 3·7) containing no species of *Pythium*. The saprophytic activity of *P. mamillatum* in soil was assessed quantitatively by determining its percentage colonization of prepared organic substrates after these had been left in contact with soil plus *P. mamillatum* inocula for 3 days. *P. mamil-*

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latum was adjudged to have colonized a particular substrate if, after this had been plated on to agar, the distinctive spiny oospores of this fungus could be found in the immediate vicinity of the substrate on the plate. Fragments of turnip storage root and portions of dead twigs and leaves of *Quercus robur* were used as substrates in these experiments.

ANTAGONISM FROM PRE-ESTABLISHED SUGAR FUNGI

Colonization of virgin v. precolonized substrates

Fragments of turnip, wood, and leaf were prepared and sterilized; half were used in this virgin condition, but the other half were left for 3 days in flasks containing soil B at 50 % m.h.c. During this time sugar fungi, together no doubt with some bacteria and members of the soil fauna, colonized the materials; since this soil was known not to contain any *Pythium* species, no complication could arise through colonization of the substrates by natural inocula of a *Pythium* species. On removal from the soil B, the fragments were brushed free from adhering soil particles. Soil A was then prepared at 50 % m.h.c. and weighed out into thirty-six flasks (50 g. in each), half of which were set up with 15 % (w/w) *P. mamillatum* inoculum mixed in with the soil. Twenty-five substrate fragments were placed in each flask, so that precolonized and virgin series of each material were distributed into soil A both at natural inoculum level and with added inoculum. The percentage of fragments colonized was determined for each flask (Table 1).

Table 1. *Percentage colonization of virgin and precolonized organic substrates*

		Natural inoculum				15 % inoculum			
		Replicate flasks				Replicate flasks			
		1	2	3	Mean	1	2	3	Mean
Turnip	Virgin	95	89	86	90	100	100	100	100
	Precolonized	0	0	0	0	28	53	28	36
Wood	Virgin	0	0	0	0	70	60	67	65
	Precolonized	0	0	0	0	18	18	15	17
Leaf	Virgin	0	0	0	0	55	72	59	62
	Precolonized	0	0	0	0	17	9	8	11

From the data obtained, it is evident that saprophytic colonization of these substrates by *P. mamillatum* is largely suppressed by a pre-established flora consisting mainly of sugar fungi. Wood and leaf pieces were not colonized by *P. mamillatum* from its natural inoculum level in soil A, irrespective of their previous treatment; this is attributed to the unfavourable nature of these substrates for colonization (Barton, 1960*b*). However, in the series in soil A with added inoculum, precolonized pieces show much reduced colonization compared with the virgin pieces. The greatest effect of precolonization was shown in turnip pieces, which in this respect would have been best for subsequent experiments. This material, however, rapidly loses its coherence when colonized by the soil flora, being difficult to wash and transfer adequately. Hence for ease of handling, wood fragments soaked in a 2% (w/v) solution of glucose were used in subsequent experiments, such substrates having been shown in preliminary tests to behave similarly to turnip pieces.

Effect of variation in length of precolonization period

An arbitrary period of 3 days in contact with soil B for the production of 'precolonized' substrate pieces was used in the preceding experiment. The present experiment was designed to test the effect of varying the period of precolonization in soil B. Sterilized wood fragments containing glucose (see above) were left in soil B for 0, 1, 2, 3, 7, and 14 days, seventy-five fragments being placed in each of five flasks with 50 g. soil B. After these periods in this soil, the seventy-five pieces were removed from each flask in its turn, and twenty-five of these were placed in each of three flasks containing soil A at its natural inoculum level. Percentage colonization of these pieces was determined after 3 days incubation (Table 2).

Table 2. *Percentage colonization of amended wood fragments after varying periods of precolonization from soil*

Days in soil B	Replicate flasks 1	2	3	Mean
0	45	44	60	49
1*	0	0	0	0

* No colonization of *P. mamillatum* was recorded for wood fragments buried in soil B for periods of 2, 3, 7 or 14 days.

A flora sufficiently antagonistic to *P. mamillatum* to prevent colonization by this fungus had developed in sterile fragments after a period of only 24 hr. in soil B. This antagonism was maintained at least to 14 days, despite any qualitative or quantitative change in the flora. Repression of *P. mamillatum* colonization by the flora developed after 1 day in soil B further suggests that sugar fungi are the chief antagonists, and also indicates that the tolerance of *P. mamillatum* to these organisms must be rather low.

Effect of nutrient level in precolonized substrates

Glucose, a nutrient source available both to *P. mamillatum* and to other sugar fungi, was used to supplement the precolonized wood fragments, the following concentrations being used: 0, 0·5, 1, 2, 5, 10, and 20% (w/v). Batches of 150 sterile wood fragments were soaked in each of these different concentrations of glucose overnight. Each batch was then incubated in 50 g. soil B at 50% m.h.c. for 3 days. On removal, the 150 fragments were divided into six series of twenty-five. Three lots of twenty-five were placed in flasks of soil A at natural inoculum level, and three lots in flasks containing soil A with added inoculum of *P. mamillatum*. The percentage of fragments colonized by this fungus was assessed (Table 3).

No colonization took place from soil A at natural inoculum level at any of the glucose concentrations used. In the 15% inoculum series, a decrease in colonization, due to the activities of the pre-established flora, can be detected when additional glucose at a concentration exceeding 1 % has been supplied.

Table 3. *Percentage colonization of precolonized wood fragments amended with different concentrations of glucose*

% glucose	15 % inoculum series*			
	Replicate flasks			
	1	2	3	Mean
0	50	68	63	60
0·5	76	71	77	75
1·0	61	76	83	73
2·0	43	64	60	56
5·0	32	68	60	53
10·0	45	50	42	46
20·0	32	55	40	42

* The zero values obtained from the natural inoculum level series are not given.

Table 4. *Percentage colonization of amended wood fragments precolonized by known sugar fungi*

	Natural inoculum				15 % inoculum			
	Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean
Wood fragments precolonized by:								
No inoculant	45	39	23	36	95	90	100	95
Trichoderma viride, 1	0	0	0	0	24	45	41	37
T. viride, 2	0	0	0	0	23	38	29	30
T. viride, 3	0	0	0	0	0	0	0	0
T. viride, 4	0	0	0	0	0	12	5	6
Penicillium frequentans	0	9	0	3	35	43	28	35
Mucor sylvaticus	0	0	0	0	46	15	39	33
M. microsporus	0	0	0	0	0	0	0	0
Rhizopus nigricans	0	0	0	0	27	33	29	30
Cephalosporium curtipes	0	0	0	0	44	13	52	36
Dicoccum asperum	0	0	0	0	60	53	69	61
Fusarium oxysporum	4	5	23	11	82	86	100	89
Sclerotial fungus	0	0	0	0	0	0	0	0

Effect of known sugar fungi separately

Because sugar fungi were chiefly concerned in suppressing colonization by *P. mamillatum* of precolonized organic fragments, some of the fungi which might be involved were isolated and their effect examined separately. Six sugar fungi were thus isolated from soil B, and six from soil A, by using glucose-amended wood fragments as baits. For convenience of handling in experiments, the twelve fungi were examined in batches of three together with the appropriate control, but the results will be presented together. The following sugar fungi were used: *Trichoderma viride* Pers. ex Fries 1, *T. viride* 2, *T. viride* 3, *T. viride* 4, *Penicillium frequentans* Westling, *Mucor sylvaticus* Hagem, *Mucor microsporus* Namyslowski, *Rhizopus nigricans* Ehrenberg, *Cephalosporium curtipes* Saccardo, *Dicoccum asperum* Corda, *Fusarium oxysporum* Schlecht. ex Fries, and a fungus which has not yet been identified since it produces only sclerotia. Each fungus was grown on 50 g. moistened mixture of sand with 3 % (w/w) maizemeal. A batch of 150 wood fragments for each fungus was autoclaved in 2 % glucose, and added to the appropriate culture 3 days after this had been inoculated. The controls consisted of autoclaved, uninoculated maizemeal-sand cultures. The 150 wood fragments were removed from the cultures after 3 days incubation at 25° C., then placed in batches of twenty-five in three flasks containing soil A at natural inoculum level, and in three flasks of soil A with 15 % added inoculum. Assessment for colonization by *P. mamillatum* was made after 3 days incubation (Table 4).

Table 4 indicates that all the twelve sugar fungi, when established separately in wood fragments, greatly suppressed saprophytic colonization by *P. mamillatum*. Least effective in this respect at the 15 % inoculum level were *Dicoccum asperum* and *Fusarium oxysporum*; this might well be due to the lower growth rate of these two fungi.

ANTAGONISM FROM PRE-ESTABLISHED FUNGI FROM OTHER SUBSTRATE GROUPS

Cellulose-decomposing fungi

Three cellulose-decomposing fungi were selected from stock cultures and tested against *P. mamillatum*. The three fungi were: *Chaetomium globosum* Kunze, *Memnoniella echinata* (Rivolta) Galloway, and *Stachybotrys atra* Corda. In view of the slow growth of cellulose-decomposing fungi, wood fragments were left in contact with inoculum for 3 days, to compare with the foregoing series, and for 17 days to ensure their complete establishment. Twelve 250 ml. flasks were prepared with 50 g. sand-maizemeal medium. Each fungus was inoculated into three flasks, this leaving three flasks uninoculated for use as controls. After 3 days, 150 substrate pieces were placed in each of the twelve culture flasks and incubated at 25° C. After 3 days incubation, one flask per fungus, together with one control flask, were taken from the incubator, the wood pieces removed and twenty-five placed in each of three flasks containing soil A, and twenty-five in each of three flasks of soil A with 15 % inoculum of *P. mamillatum*; the percentage of fragments subsequently becoming colonized was recorded. After 17 days in the incubator, a similar test was set up using four of the remaining culture flasks. The final four flasks were also used in a similar manner, but, in this case, the wood fragments, on removal from the pure cultures, were allowed to stand in a 2 % glucose solution for 24 hr. This latter treatment was included, in case the original glucose had, over the 17-day incubation period, diffused or been removed in some other way from the fragments. The complete data resulting from this experiment are given in Table 5.

Table 5 shows that, over the shorter incubation period giving 3 days for precolonization, the cellulose-decomposing fungi had entered the wood fragments and had subsequently repressed saprophytic colonization by *P. mamillatum*. *Chaetomium globosum* was most effective as an antagonist; its rapid growth rate probably allowed more rapid colonization. Similarly, after 17 days incubation all three cellulose-decomposing fungi had become well established in the fragments and all consequently limited colonization by *P. mamillatum*. With all three fungi, the additional glucose treatment of the wood fragments resulted in a reduction of percentage colonization by *P. mamillatum*. This effect has been noted above with sugar fungi, where additional glucose in the substrate stimulated activity of the pre-established fungi.

Table 5. *Percentage colonization of amended wood fragments precolonized by known cellulose-decomposing fungi*

	Natural inoculum				15 % inoculum			
	Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean
Wood fragments precolonized by:	3 days precolonization							
No inoculant	64	46	55	55	93	90	95	93
Chaetomium globosum	0	0	0	0	0	13	0	4
Memnoniella echinulata	0	5	0	2	26	43	25	31
Stachybotrys atra	0	10	0	3	26	39	25	30
	17 days precolonization (no glucose)							
No inoculant	47	36	34	39	69	75	78	74
Chaetomium globosum	0	0	0	0	45	41	50	45
Memnoniella echinulata	0	5	5	3	46	65	47	53
Stachybotrys atra	0	0	0	0	45	63	55	54
	2 % glucose added							
No inoculant	37	36	43	39	71	74	90	78
Chaetomium globosum	0	0	0	0	36	18	33	29
Memnoniella echinulata	0	0	0	0	31	38	40	36
Stachybotrys atra	6	11	0	5	43	26	47	39

Table 6. *Percentage colonization of amended wood fragments precolonized by known lignin-decomposing fungi*

	Natural inoculum				15 % inoculum			
	Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean
Wood fragments precolonized by:	3 days precolonization							
No inoculant	50	62	65	59	95	100	100	98
Armillaria mellea	61	64	79	68	95	100	100	98
Fomes annosus	0	0	0	0	0	0	0	0
Merulius lacrymans	57	55	63	58	100	100	100	100
	31 days precolonization (no glucose)							
No inoculant	35	26	38	33	88	72	80	80
Armillaria mellea	0	0	0	0	33	48	47	43
Fomes annosus	0	0	0	0	35	37	23	32
Merulius lacrymans	0	0	0	0	28	50	54	44
	2 % glucose added							
No inoculant	38	62	52	51	100	100	100	100
Armillaria mellea	0	0	0	0	8	0	0	3
Fomes annosus	0	0	0	0	0	9	0	3
Merulius lacrymans	0	0	0	0	17	10	0	9

Lignin-decomposing fungi

The following three lignin-decomposing fungi were selected from stock cultures: *Armillaria mellea* (Vahl ex Fr.) Kummer, *Fomes annosus* (Fr.) Cooke, and *Merulius lacrymans* (Wulf.) Fr. This experiment was set up in the same way as the preceding one, except that a 31-day incubation period was substituted for the 17-day one (Table 6).

It is evident from Table 6 that after 3 days in contact with lignin-decomposing fungi, only *Fomes annosus* had colonized the fragments, *P. mamillatum* being prevented from colonizing only in this series. The percentage of fragments colonized in the other treatments was similar to that in the control. After 31 days, most wood fragments in all the treatments had become colonized by the respective lignin-decomposing fungi, and all three fungi had reduced colonization by *P. mamillatum* to a considerable extent. With added glucose, the previously recorded effect of a reduction in percentage colonization was obtained.

NATURE OF THE ANTAGONISTIC MECHANISM

The antagonistic mechanism operating between the organisms in the organic substrate and *P. mamillatum* has been the subject of a previous paper (Barton, 1960). Briefly, it was suggested that three alternative antagonistic mechanisms might be involved, namely, lack of nutrient, lack of available space, or interplay of toxins, i.e. specific antibiotics or, alternatively, general staling substances. Experimental evidence was presented from which it was inferred that the antagonism was due to the production of ordinary staling substances by the pre-established fungi.

Microscopic observation of colonization

Glass-fibre tapes which had been left for periods of 1 week and 1 month on oat agar plates, and containing oospores, chlamydospores, and resting sporangia, were used to represent inocula of *P. mamillatum* which might normally be found in soil (Barton, 1958). Turnip pieces were sterilized, and two-thirds of these placed in soil B for 3 days. This precolonized material was then treated in two ways, one series being soaked in 2% glucose for 2 hr. and the remainder used directly. In all three treatments, i.e. sterile, precolonized, and precolonized plus glucose, the pieces were placed on triplicate colonized tapes buried in soil A contained in Petri dishes. Tapes were removed after 3 days, stained in steaming phenolic rose bengal and mounted in water. Sterile turnip fragments caused a similar response in both 3-day- and 1-month-old *P. mamillatum* inocula. Corresponding with the positions of the turnip material on the tapes, hyphal development from *P. mamillatum* propagules was so dense as to cover the tape in that region and extend into the surrounding soil. The treatments involving precolonized fragments contrasted markedly with the above; there was no hyphal activity at all from the resting bodies of *P. mamillatum*. A similar result was also obtained in the series involving precolonized fragments plus glucose. These findings, therefore, confirm the results obtained by indirect techniques, namely, that precolonized organic material is not colonized by *P. mamillatum*. Furthermore, the fact that the inoculum remains inactive suggests that an inhibitory toxin of some kind is involved, since the fragments soaked in glucose, i.e. with suitable nutrient at the surface of the material, did not increase *P. mamillatum* activity. Also, the absence of stimulation does not favour the hypothesis that *P. mamillatum* is prevented from entering the material by lack of available space. If this were the only antagonistic mechanism, then the inoculum of *P. mamillatum* would be expected to become active, and only later be prevented from entering by a barrier of hypha.

Growth of Pythium mamillatum *in staled media from selected sugar fungi*

The twelve sugar fungi isolated from soil and used in a previous experiment were grown individually for periods of 1 and 2 weeks in Czapek's solution with 2 % glucose. The staled medium after growth of each fungus was collected through a Seitz filter, part being dispensed into three sterile 100 ml. flasks, and the remainder retained for pH determination. *P. mamillatum* was finally inoculated into each flask by means of a marginal disk cut from a colony on oat agar, and incubated at 25° C. Dry weight of the mycelium produced during 2 weeks growth was determined after drying at 100° C. for 3 hr. (Table 7).

Table 7. *Growth of* Pythium mamillatum *in staled media from various sugar fungi*

	7 days previous growth of antagonist		14 days previous growth of antagonist	
	Dry weight of *P. mamillatum**	pH	Dry weight of *P. mamillatum**	pH
Unstaled medium	60·0	6·95	54·9	6·95
Trichoderma viride, 1	47·2	7·4	15·7	7·4
T. viride, 2	2·5	7·2	10·0	7·3
T. viride, 3	33·2	7·8	18·3	7·5
T. viride, 4	32·4	7·7	2·8	7·1
Penicillium frequentans	9·7	6·5	7·3	7·4
Mucor sylvaticus	41·5	6·6	23·1	6·8
M. microsporus	27·6	6·8	3·5	7·8
Rhizopus nigricans	13·1	7·1	4·0	6·0
Cephalosporium curtipes	26·0	7·6	4·1	7·6
Dicoccum asperum	43·4	7·5	31·9	7·7
Fusarium oxysporum	22·5	6·8	2·4	7·4
Sclerotial fungus	21·5	6·5	0	6·9

* Figures for mycelial dry weights are in mg., and are means of three replicates.

Table 7 shows that when *P. mamillatum* was inoculated into staled media, growth was poor compared with that in fresh medium. The degree of inhibition varied with the different fungi involved, but, except in the case of *T. viride* 2, was greater after 14 than after 7 days staling of the medium. Change in pH of the medium was caused by all the fungi, but in no instance was this adjudged sufficiently great to result in the observed inhibition of growth by *P. mamillatum*. All the sugar fungi, therefore, tended to produce metabolic products in liquid culture inhibitory to the vegetative growth of *P. mamillatum*. Nevertheless, these same toxins, when impregnated into sterile wood fragments, did not affect subsequent colonization by *P. mamillatum* (Barton, 1960).

Evidence from strains of Trichoderma viride

Experiments were carried out with three different strains of *Trichoderma viride* differing in their ability to produce antibiotics. Strain 211 produced the antibiotic gliotoxin; strain 941 produced viridin; strain 939 did not produce either antibiotic (the strain numbers are those given by the Akers Research Laboratories, Welwyn). First, the three strains were grown in sand-maizemeal culture for 3 days, and then 150 pieces of amended wood added to each culture and to a control flask which remained uninoculated. The fragments were removed from each treatment after 3 days, half being placed in sets of twenty-five in three flasks containing soil A and the other half in three flasks containing soil A plus 15% inoculum of *P. mamillatum*. Percentage colonization of the wood fragments by *P. mamillatum* in each flask was recorded. Secondly, the

Table 8. *Behaviour of* Pythium mamillatum *towards strains of* Trichoderma viride *differing in their ability to produce antibiotics*

(*a*) *Percentage colonization of amended wood fragments precolonized by different strains of* T. viride

	Natural inoculum				15% inoculum			
	Replicate flasks				Replicate flasks			
Strain of *T. viride*	1	2	3	Mean	1	2	3	Mean
No inoculant	30	18	16	21	48	63	65	59
Gliotoxin producer	0	0	0	0	0	0	0	0
Viridin producer	0	0	0	0	0	0	0	0
Non-producer	0	0	0	0	15	0	4	6

(*b*) *Percentage colonization of wood fragments impregnated with staled media from strains of* T. viride

	Natural inoculum				15% inoculum			
	Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean
No inoculant	45	42	47	45	100	95	100	98
Gliotoxin producer	0	0	0	0	70	79	55	68
Viridin producer	0	0	0	0	78	61	57	65
Non-producer	48	40	41	43	100	86	95	94

(*c*) *Growth of* P. mamillatum *in staled media from strains of* T. viride

	Mycelial dry weight (mg.)	pH
No inoculant	60·8	6·9
Gliotoxin producer	No growth	7·8
Viridin producer	5·4	7·8
Non-producer	6·7	7·3

strains of *T. viride* were cultured for 14 days in modified Czapek's solution and the staled growth media collected through a Seitz filter. Sterile wood fragments were allowed to soak in each batch of staled medium, and in fresh medium, for 24 hr. The wood fragments were then placed in sets of twenty-five in two series of triplicate flasks, containing, respectively, soil A, and soil A plus 15 % *P. mamillatum* inoculum. Thirdly, the staled media collected from the different strains of *Trichoderma viride* was dispensed in 10 ml. portions into triplicate flasks, which were inoculated with *P. mamillatum* and incubated at 25° C. for 2 weeks. The staled medium remaining was used for pH tests. The complete data are given in Table 8.

Percentage colonization of wood fragments by *P. mamillatum* was drastically reduced when any of the three strains of *Trichoderma viride* was first established in the substrate. There was, however, a low percentage colonization of the fragments containing the strain not producing antibiotic. When the wood fragments were impregnated with stale medium, a clear distinction between the effect of the antibiotic-producing strains and that having no antibiotic could be drawn. Where viridin or gliotoxin had presumably been liberated into the medium, colonization of impregnated wood fragments was markedly reduced. Where no antibiotic had been produced, and the medium contained only general staling substances, impregnated wood fragments were colonized to an extent not very different from that found in fragments impregnated with fresh medium. Finally, growth of *P. mamillatum* in filtered, staled media was inhibited by all three strains of *T. viride*, although there was slightly better growth in the medium from the non-antibiotic-producing strain. From these results, it is evident that specific antibiotics, which remain potent at extreme dilution, can prevent colonization of impregnated wood fragments by *P. mamillatum*. Other, more general, staling substances, though liberated into the growth medium in sufficient quantity to be inhibitory to *P. mamillatum* in culture, are nevertheless non-inhibitory when impregnated into wood fragments, presumably due to volatilization, inactivation or adsorption.

Evidence from known antibiotics

Six antibiotics, gliotoxin, viridin, patulin, mycophenolic acid, frequentin, and griseofulvin were made up to standard solutions of 100 μg./ml. in 2 % glucose solution. The following dilutions were then prepared from this, using 2 % glucose solution as diluting agent: 5, 25, 50, and 100 μg./ml. Controls containing 2 % glucose only were also set up. Batches of 150 wood fragments, after autoclaving in distilled water, were placed in each of the four concentrations of each antibiotic and allowed to soak for 24 hr. On removal, three lots of twenty-five fragments were placed in triplicate flasks of soil A, and three lots of twenty-five in triplicate flasks of soil A plus 15 % inoculum of *P. mamillatum*. The percentage of wood fragments colonized after 3 days was recorded (Table 9).

Table 9 shows that griseofulvin had little inhibitory effect even at concentrations of 100 μg./ml., but Brian (1949) has shown that this substance is inhibitory only to fungi having chitinous cell walls, so that

Oomycetes, such as species of *Pythium*, remain unaffected. The other five antibiotics all inhibited colonization by *P. mamillatum* but to varying extents. For instance, at 5 μg./ml. gliotoxin and frequentin were not greatly inhibitory, some colonization occurring even at 25 μg. with the latter. With the other three antibiotics, concentrations of 5 μg./ml. drastically reduced colonization.

Table 9. *Percentage colonization of wood fragments impregnated with different concentrations of antibiotics*

		Natural inoculum				15 % inoculum			
		Replicate flasks				Replicate flasks			
	Conct. (μg./ml.)	1	2	3	Mean	1	2	3	Mean
Griseofulvin	0	32	24	29	28	65	59	55	59
	5	27	43	28	33	55	71	80	68
	25	33	33	33	33	65	57	59	60
	50	25	29	35	29	67	77	55	66
	100	41	26	27	34	76	72	59	69
Gliotoxin	0	30	32	30	31	48	45	50	48
	5	27	27	30	28	35	39	36	37
	25	0	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0
Viridin	0	30	32	30	31	48	45	50	48
	5	9	10	0	6	9	5	9	8
	25	0	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0
Patulin	0	40	50	50	47	81	85	77	81
	5	0	9	5	5	40	33	43	38
	25	0	0	0	0	0	16	0	5
	50	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0
Mycophenolic acid	0	40	50	50	47	81	85	77	81
	5	0	5	15	7	17	26	21	21
	25	0	0	0	0	0	0	9	3
	50	0	0	0	0	10	0	0	3
	100	0	0	0	0	0	0	0	0
Frequentin	0	40	50	50	47	81	85	77	81
	5	42	27	39	46	75	65	68	69
	25	9	0	0	3	10	16	24	17
	50	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0

DISCUSSION

Experiments to ascertain the ability of *P. mamillatum* to colonize soil substrates already having a fungus flora showed clear-cut results, namely, that this fungus was unable to establish itself. *P. mamillatum*, therefore, and possibly other species of *Pythium* also, must be restricted in soil to colonization of virgin substrates only. Moreover, *P. mamillatum* is also limited to soil substrates containing an available carbon source (Barton, 1960*b*). These two factors, then, would curtail the saprophytic activity of *P. mamillatum* in soil, probably to an extent not hitherto realized, and, in addition, would influence both seasonal variation in activity, and the distribution in soils of members of this genus. Thus the habitats where fresh virgin substrates are most constantly being added to soil are cultivated soils; in woodland soils and those of other natural habitats, a proportion of the organic substrates falls from the aerial parts of plants, and already possesses a typical flora of aerially disseminated saprophytes and weak parasites (Webster, 1956, 1957; Hudson & Webster, 1958; Pugh, 1958). This may well be one of the chief reasons why species of *Pythium* are pre-eminently fungi of cultivated soils (Chesters, 1949; Barton, 1958).

P. mamillatum in particular, and most other species of *Pythium* in general, show certain features concomitant with their position as pioneer colonizers. Garrett (1956) has pointed out that initial colonization of organic material is analogous to a race, and that certain physical characteristics lead to success in this ecological niche. Thus *P. mamillatum* has an extremely high growth rate, which can be attributed, at least on agar, to the rapid extension of sparsely distributed, radial runner hyphae. This attribute is particularly important for penetration of organic substrates, or for growth through soil to reach other suitable material. Given the necessary physical characteristics for occupying such a pioneer ecological niche, the question arises as to how *P. mamillatum* exploits the advantage thus gained. In situations such as this, the adage of 'First come, first served' certainly holds true, so that the pioneer colonizer obtains an initial position for development ahead of its competing rivals, which should give the fungus an advantage in persisting in the colonized tissue. Garrett (1940, 1956) has used the term 'saprophytic survival' to denote the persistence of viable hyphae in a state of slow activity in saprophytically colonized tissues, and has been followed in this by Butler (1953*b*). In the case of *P. mamillatum*, saprophytic survival in this proper sense is not evident. One or two days after the fungus has permeated the substrate, the hyphal contents are utilized in the formation of resting bodies, and it is thus this inactive form which survives in organic material, and in the soil when finally released by the decay of the tissues. Exploitation of this pioneer position by *P. mamillatum* also includes the outwards radial growth of hyphae through and over soil. Such growth tends to bring more material into the range of colonization, by spatially increasing the extent of inoculum in soil, and also by effectively increasing the inoculum potential of the fungus relative to the original condition of the inoculum.

The antagonism between sugar fungi, established in organic material in soil, and *P. mamillatum* has been ascribed to the production of ordinary staling substances by the former. The extent to which these implications can be applied to other fungi in a similar ecological situation depends largely on their relative tolerance to such external metabolites as compared with *P. mamillatum*. There is little doubt that *Pythium* species in general are relatively intolerant of antibiotics (Gilliver, 1946) and other staling substances. The distinction between two 'levels' of antagonism from metabolic products therefore is quite likely to be a property specific to this fungus. More tolerant organisms might not be so sensitive to general staling substances, and antagonism would first be detected at the specific antibiotic 'level'. It should be possible therefore, assuming this were true, to arrange fungi in a series in order of increasing tolerance to external metabolic substances. From such a series, the prediction of a succession of sugar fungi on organic material might be possible, since successive colonizers would have to overcome the increasing antagonism produced in the organic material. Thus a fungus like *P. mamillatum* at the head of the series, having least tolerance to antagonism, would be confined to pioneer colonization. For fungi with greater tolerance, the organic material would still be available even though it possessed some antagonism, and these fungi would be found in the later stages of succession. Garrett (1956) has suggested four attributes which make for competitive saprophytic ability: rapid growth rate and rapid germinability of spores, good enzyme-producing equipment, production of antibiotic toxins, and tolerance to antibiotics produced by other organisms. Of these attributes, tolerance to antibiotics has been considered of superior importance for saprophytic ability under the antagonistic conditions existing in soil (Anwar, 1949; Butler, 1953*a*; Park, 1956). For *P. mamillatum* an outstanding characteristic is a low tolerance of antagonism, yet, paradoxically, this fungus is an extremely successful soil saprophyte. However, colonization of virgin substrates can also be regarded as an escape from antagonism, in that colonization only occurs when non-antagonistic situations, as in virgin substrates, are available to an inoculum of the fungus. It is factors favourable to this escape mechanism, therefore, which are prominent in this group, namely, 'quickness off the mark', and subsequent rapid hyphal growth. Similarly, the characteristic production of resting bodies may also be regarded as part of this escape mechanism, in that it is a rapid change from an intolerant to a tolerant condition.

From what has gone before, it becomes apparent that the term 'sugar fungi', encompassing such a vast array of soil fungi, could profitably be divided further. Of the fungi placed in this category, certain saprophytes, here exemplified by *P. mamillatum*, function as pioneer colonizers of any available substrate, and can occupy no other place in the ecological succession. These fungi should clearly be delimited from the main group of sugar fungi. Possibly the term 'pioneer sugar fungi' might be specifically applied to this subgroup.

I wish to thank Prof. C. W. Wardlaw for supervising this work, and Dr D. Park for much helpful criticism. Dr P. W. Brian kindly supplied strains of *Trichoderma viride*, and samples of specific antibiotics. Finally, thanks are due to the Agricultural Research Council for the award of a Research Scholarship.

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ISOLATION OF *FUSARIUM OXYSPORUM* FROM SOILS

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A method for the isolation of *Fusarium oxysporum* is described which is sensitive to populations smaller than 10 units/g. soil.

In an examination of British soils for the presence of *Fusarium oxysporum* Snyder & Hansen, four different isolation methods have been used that are known to be more sensitive for this species than is the standard method of dilution plating. None, however, has proved to be completely reliable. Of thirty-five soils examined by these methods, fifteen were shown to contain *F. oxysporum*. Plating washed portions of roots (Harley & Waid, 1955) of pea grown in the soils gave positive results from fourteen of them, roots of flax from seven, and direct droplet plating and droplet plating of grass baits (Park, 1958) from eight soils each. These differences could have resulted from physiologically different populations of the fungus in the different soils but there is evidence against this. Thus the soils that had not given *F. oxysporum* by direct droplet plating but had by pea-root plating were re-examined by droplet plates after having had peas grown in the soil, when five of the six showed the fungus this time. A further thirty-three isolates of *F. oxysporum*, including some from those soils that had not given flax root infestation, some from tropical soils, and some from wilted banana and oil-palm plants, were examined for the ability to infest the surface of flax roots in artificially inoculated soil, and all proved successful at this. It seems probable that it was the sometimes low population level of this species in soil that had led to unreliable results by the methods used. The four methods have been compared in their sensitivity to known levels of *F. oxysporum* in artificially inoculated soils, and it was found that 100 units/g. soil was necessary for good infestation of flax roots and also for reliable detection of the fungus by the direct droplet method, using fifty inoculated droplets. At 10 units/g. soil the grass-bait method was reliable with fifty plated baits, but at lower levels the method gave variable results. Pea roots detected the fungus well at levels as low as 1 unit/g. soil, but this method has the disadvantage for certain purposes of possible selectivity for certain forms only of *F. oxysporum*. Moreover, for general purposes baiting methods can be criticized in that they require not only the presence of the fungus but also its activity under the conditions given. Baiting is also more time-consuming than direct plating, increases the risk of contamination from outside, and itself affects the population level of the fungus in the sample.

Standard methods of direct plating either of soil (Warcup, 1950) or of diluted suspensions of soil (Waksman, 1927) would require more than fifty replicate dishes from each sample reliably to show the fungus from a soil population of 100 units/g. Yet for the ecology of *F. oxysporum* much lower levels than this are significant. It was pointed out above that 1 unit/g. is sufficient to give good infestation of pea roots, and Rishbeth (1955) states that *F. oxysporum* f. *cubense* populations smaller than 2 units/g. soil can lead to wilt in banana. The author has attempted to evolve a method of direct plating that is sensitive to populations smaller than 10 units/g. soil.

Description of method

The method is based on the known high tolerance of *F. oxysporum* for high concentrations of carbon dioxide, the aim being to reduce the total number of species able to develop on a plate, thus allowing the quantity of soil inoculum to be increased, and thereby increasing the sensitivity of the method for those species still capable of developing. The following technique has been found workable. For one soil sample, five sterilized Petri dishes are each inoculated with 0·2 g. soil (air-dry equivalent). Melted malt-extract agar (20 ml.) at 42° C. is poured into each dish and the soil dispersed through the agar. When cooled, the plates are stacked in a strong-walled closeable vessel with controlled inlet and outlet cocks—a desiccator has been used here. The outlet leads through a mercury manometer to a suction pump; the inlet to a cylinder of carbon dioxide. With the inlet closed and the outlet open the container is evacuated to 60 cm. column of mercury. Then with the outlet closed and the inlet open carbon dioxide is let in until atmospheric pressure is reached in the container. This process is repeated twice more, both taps are then closed, and the container is incubated at 30° C. for 8–10 days. After this time the dishes are taken out of the container and placed under normal bench conditions for 1–3 days, when colonies of *F. oxysporum* are readily detected.

The triple evacuation of the container and replacement of removed gases by carbon dioxide has been found by measurement to give an atmosphere of just over 99 % carbon dioxide. Sixty-one available isolates of *F. oxysporum* have been grown under these conditions in pure culture at 30° C. and all developed a normal colony morphology, producing pigment some hours after their return to bench conditions. The thirty-five soils investigated using the other methods have been re-examined for *F. oxysporum* by this method. All those that had given the fungus by any of the earlier methods also gave it here, and one soil that the survey had not shown to contain the fungus gave it by this method.

A full account of the variations on the method that have been attempted would be of little value, yet some comments on relevant points might be useful. Replacement of withdrawn gases by nitrogen instead of carbon dioxide did not give good results, a larger number of species developing on the plates. The positive inhibitory action of carbon dioxide seems to be more important than the reduction in oxygen tension. Lower concentrations (around 80 %) of carbon dioxide allow more rapid development of *F. oxysporum* but also allow a greater number of other species to develop, giving less-reliable results where *F. oxysporum* is present in low numbers. All sixty-one *F. oxysporum* isolates tested were able to grow in carbon dioxide at the higher temperature of 33° C. but development here was inconveniently slow. At temperatures below 30° C. there was interference from a greater number of other species, some of which spread rapidly, making isolation of *F. oxysporum* more difficult. At 30° C. the most common fungi appearing on the plates were *F. oxysporum*, *F. solani*, *Mucor racemosus*, and species of *Paecilomyces*, *Aleurisma* and *Arachniotus*. *Trichoderma viride* appeared more rarely. Only *M. racemosus* grew at all rapidly.

For this method the ratio of soil to agar is important. In an attempt to reduce the space required during incubation, the soil inoculum was added to the surface of agar in slopes. Most soils gave no fungal growth within 2 weeks under the incubation conditions detailed above. This is interpreted as indicating that, in isolation methods such as this, the agar in addition to providing nutrients also dilutes soil toxicity for the fungi, and that carbon dioxide has some effect on this relationship. In Petri dishes poured with 20 ml. agar, inocula of 0·1 to 0·3 g. soil gave good results, but 0·5 g. often led to poor and irregular growth; 0·2 g. soil/dish was therefore adopted as a convenient standard.

Table 1. *Check on sensitivity of carbon-dioxide plating method for* Fusarium oxysporum

Added conidia/g. soil	Colonies *F. oxysporum*/dish	Estimated units/g. soil
1	2; 1; 1; 1; 0	5
10	5; 3; 4; 2; 1	15
100	26; 21; 17; 15; 13	92

Some cultivated soils when used with this method show a tendency to 'blow' the agar, causing it to crack away from the base of the dish. Fungal growth is inhibited in such dishes. Putting the soil inoculum on instead of under the agar prevented the 'blowing', but bacterial development and the consequent inhibition of fungal growth still occurred. The phenomenon is thought to be caused by bacterial fermentation, probably by species of *Clostridium*. *F. oxysporum*, when present, will eventually show itself on such 'blown' plates but only after a longer and less convenient (up to 12 days) post-carbon-dioxide incubation. Furthermore, the smell of butyric acid may be objectionable. Rose bengal agar (Smith & Dawson, 1944), but not acidified agar (e.g. that used by Warcup, 1950), overcame this disadvantage in those soils that show it. With some experience *F. oxysporum* may still be directly recognized on this agar, which also prevents the spread of *Mucor racemosus*.

The sensitivity of the method in practice has been checked by plating artificially inoculated soil. Conidial suspensions of a soil isolate of *F. oxysporum* were added to a soil not containing any indigenous *F. oxysporum* to give calculated levels of 1, 10, and 100 spores/g. soil. Table 1 shows that at the lower levels the estimates of numbers obtained by the use of this method are considerably higher than those calculated. This trend has been found in two repetitions of the check. Nevertheless, it is clear that the fungus can readily be detected at population levels below 10 units/g. soil. More replicate plates would be necessary for detection of populations below 1 unit/g. soil. The method can be used without modification for quantitative determinations of the population of *F. oxysporum* in soils.

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A NOTE ON VARIATION BETWEEN ISOLATES OF *ARMILLARIA MELLEA* (VAHL EX FR.) KUMMER

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(With Plate 10 and 2 Text-figures)

In the course of studies of disease caused by *Armillaria mellea* in pine plantations in Kenya, an attempt was made to compare local isolates of the fungus with others obtained from various parts of the world. The morphology and temperature relationships of 140 such isolates, of which 38 were obtained in East Africa, have been studied. The remainder, including four isolates of the closely related *Clitocybe tabescens*, were obtained through the generosity of mycologists elsewhere.

Differences in morphology of *Armillaria mellea* in culture

Isolate morphology was studied in cultures grown on a 2 % malt agar medium to which 0·05 % of Difco yeast extract was added. The agar was sloped in 6 × 1 in. boiling tubes and the cultures were incubated for 3 weeks in a glass-fronted cupboard at room temperature (16–20° C.). Growth was then recorded according to the following system.

Mycelium. A thin crustose skin was formed to a greater or less extent by all isolates, of which the aerial mycelium took various forms. These forms were classified as follows:

A. Aerial mycelium present in small tufts not more than 1·5 mm. high, coloured from orange through brown tints to brown.

B. Aerial mycelium very sparse, not markedly tufted.

C. Aerial mycelium present as large tufts more than 1·5 mm. and up to 4 mm. high, coloured from orange to pinkish brown or brown.

D. Aerial mycelium thick and nearly uniform, white, sometimes with yellow tints. Crustose skin in these cases very thin. Diagrams of these mycelial types are shown in Text-fig. 1.

Rhizomorphs. Rhizomorphs have been classed on their vigour and, in the most vigorous growths (type 3), have been further subdivided according to their morphology. Vigour of growth was classified under three heads:

(1) Rhizomorphs absent or produced only to a very restricted degree, never exceeding the radius of the mycelial mat.

(2) Rhizomorphs of moderate vigour but with a radius of growth never exceeding twice that of the mycelial mat (Text-fig. 2 (2)).

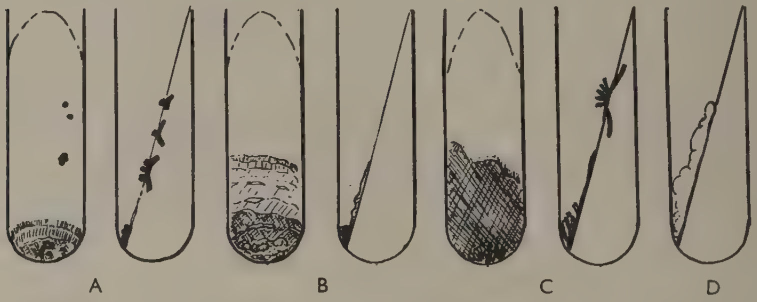
(3) Rhizomorphs growing vigorously, extending to more than twice the radius of the mycelial mat. These rhizomorphs fell into four morphological groups:

(*a*) Thin rhizomorphs, 1–2 mm. diam., cylindrical in section, brown to dark brown.

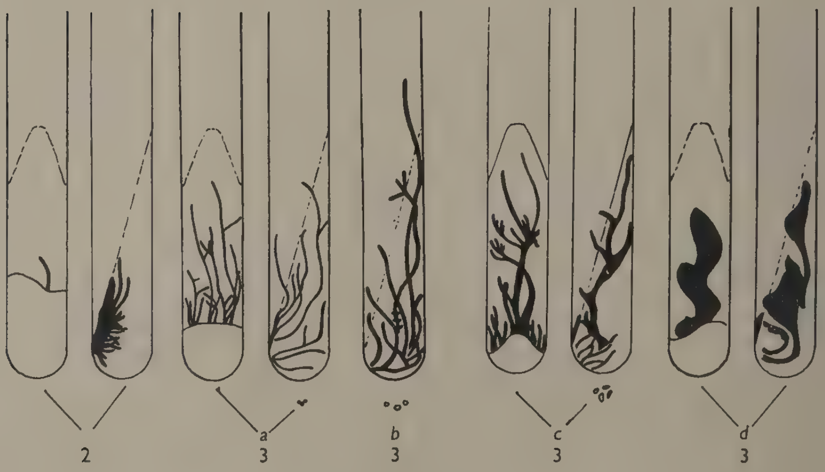
(*b*) Rhizomorphs thicker than in (*a*), up to 5 mm. diam., uniform and cylindrical, tending to give rise to aerial rhizomorphs directly from the seat of the inoculum in culture, various shades of brown.

(*c*) Thick rhizomorphs, 3–5 mm. wide, tending to be flattened in cross-section, dark brown to almost black. They sometimes tend to emerge from the agar but are never wholly aerial structures.

(*d*) Flattened fan-like growth, cream to light brown in colour.



Text-fig. 1



Text-fig. 2

Diagrams of these rhizomorph types are given in Text-fig. 2; types (3) (*a*), (*b*) and (*c*) are illustrated in Pl. 10, figs. 1 and 2.

Pigment. The production of brown pigment into the agar medium varied considerably but was classed under only two heads, 'absent or very slight' and 'present'.

Luminosity. This was assessed in conjunction with experiments to determine the effects of nutrient concentration (see below). At the end of each nutrient trial, the full set of tubes was taken into a photographic dark room and after 5–10 min. the lowest nutrient value at which luminosity was observed was noted.

Morphology in relation to origin of isolate. The majority of isolates were found to be reasonably constant in form throughout successive subcultures. One isolate obtained from India (Himalayas) apparently sectored in a plate culture and subcultures from each variant maintained their character in subsequent cultures. The only isolate to show rhizomorphs of type (*d*) proved unstable and in later subcultures reverted to the production of rhizomorphs of moderate vigour, type (2). Three East African isolates which originally showed only poor rhizomorphic growth of type (1), later increased in vigour but did not change otherwise. The original forms and reactions of these isolates will be discussed below.

Little correlation was found between morphological type and substrate of origin, or time maintained in culture. However, six isolates that had been kept in culture for 15 years or more showed little or no rhizomorph growth, suggesting that protracted culture tends to reduce this feature of growth type.

Table 1. *Variation in morphology in relation to geographical origin of isolates of* Armillaria mellea

	Distribution of mycelial and rhizomorphic types (numbers of isolates)									
	Mycelial types					Rhizomorphic types				
	A	B	C	D	Total	*a*	*b*	*c*	*d*	Total
Europe	14	10	0	17	41	21	0	0	0	21
Asia	6	1	1	0	8	1	0	0	0	1
Africa	36	3	0	5	44	0	0	33	1	34
N. and S. America	6	2	1	4	13	1	1	0	0	2
Australia and New Zealand	12	1	12	5	30	2	11	0	0	13
Totals	74	17	14	31	136	25	12	33	1	71

Some degree of segregation of morphological types according to continent of origin was found (Table 1). Mycelium of type C was found in isolates from Australia and New Zealand in much higher proportion than in those from elsewhere. Type (*a*) rhizomorphs were found in cultures of European origin, whereas type (*b*) occurred in isolates from Australia and New Zealand and was frequently associated with aerial mycelium of type C. Type (*c*) rhizomorphs occurred in African isolates.

The degree of luminosity and pigment production in agar did not appear to be related to origin of an isolate or to any other of the characters studied. Out of 136 cultures, 90 were found to be luminous.

Effects of nutrient concentration on growth and morphology

These effects were studied using an agar medium (2 % agar) containing 'Oxoid' malt extract at the following concentrations: 0, 0·2, 1·0, 2·0, 5·0 and 8·0 %. Aliquots of 7 ml. were run into 5 × ½ in. tubes and allowed to set with the tube vertical, as shown in Plate 10, fig. 2. This followed a method used by Bliss (1946), and permitted a maximum depth of agar down through which the rhizomorphs could grow. Small pieces of mycelium cut from 2 % malt agar cultures, approximately 3 mm. square, formed the inocula for all tests. For each concentration of malt extract, three tubes were inoculated with each isolate of *A. mellea* and incubated at 25° C. for 25 days. At 5-day intervals, the length and number of rhizomorphs formed were recorded and a note made of pigment production. At the end of the experiment, luminosity was observed.

Growth rate, luminosity and pigment production increased with increasing nutrient concentration for all isolates tested. The greatest increases occurred in the range from 0 to 2·0 % malt extract, with correspondingly smaller margins of response at the 5·0 and 8·0 % levels. These increases were never so great as to alter the morphological status of an isolate within the classification used. Under these conditions of growth, rhizomorphs were generally very pale in colour. Where luminosity occurred, it was usually apparent in growths at the 2 % level of malt extract; however, seven isolates were found only to be luminous when grown in 5·0 and 8·0% malt agars. As far as it was possible to judge, pigment production seemed to increase in direct proportion to nutrient concentration.

EFFECT OF TEMPERATURE ON GROWTH RATE OF ISOLATES

Temperature effects were investigated by culturing the isolates on 2 % malt agar slopes in triplicate at 15, 22–24, 25–27, 27–29, 30–32 and 32–35° C. Exact control of temperatures was not possible with the apparatus available. Notes were taken of mycelial growth and the length and number of rhizomorphs formed every 5 days over a period of 25 days. A total of 116 isolates was tested.

The distributions of optimum temperatures for mycelial and rhizomorph growth follow the same pattern closely, whether the isolates were obtained from tropical or temperate sources (according to latitude of origin). These agree with the values obtained by Wolpert (1924), Reitsma (1932), Benton & Ehrlich (1941), Bliss (1946) and Townsend (1954). Results are shown in Table 2; only three temperatures are given as no optima were observed at 15, 30–32 or 32–35° C. With a fungus as variable as *A. mellea*, some variation in optimum temperatures between isolates is to be expected. However, no correlation between optimum temperature and the climate of origin of the isolate has been made out, either on the basis of subclassification according to latitude (Table 2) or from more detailed comparisons between individual isolates. No correlation has

Table 2. *Optimum temperatures for mycelial and rhizomorphic growth of* 116 *isolates of* Armillaria mellea *from temperate and tropical environments*

		Distribution of temperature optima (numbers of isolates)		
		22–24° C.	25–27° C.	27–29° C.
Temperate	Mycelium	19	25	9
	Rhizomorphs	12	20	8
Tropical	Mycelium	27	29	7
	Rhizomorphs	14	28	4

been found between temperature relationships and rhizomorph morphology, or continent of origin.

Selected isolates were also tested for tolerance to temperatures below 0° C., and to temperatures higher than those permitting mycelial growth but which were likely to occur in tropical environments. Low temperature tests were made by placing freshly inoculated slopes of 2 % malt agar in the ice chamber of a refrigerator for periods up to 2 months, followed by incubation at 25° C. to determine viability. Tolerance to high temperatures was tested in a similar way. Freshly inoculated slopes of 2 % malt agar were exposed to temperatures of 39 and 45° C. for periods of 5 and 30 min. and then incubated at 25° C. to test for renewal of growth.

In both these series of experiments, only a limited series of isolates was used, obtained from tropical (Sao Thome Islands and Yangambi, Belgian Congo) temperate (Kenya Highlands; Azores; Victoria, Australia) and low temperature (Norway; Sweden) regions. Again, all isolates tolerated high and low temperatures and no differences were noted between those from widely different environments.

CLITOCYBE TABESCENS

Four isolates of *Clitocybe tabescens* were included in the collection of isolates studied. Their growth in culture was very similar to that of certain isolates of *A. mellea*. One isolate differed from Rhoads's (1945) description of the fungus in culture by being luminous. Another formed a small but perfect sporophore, which Rhoads has described as characteristic of the species. It is interesting to note that a single sporophore of *A. mellea*, the only instance of success from many attempts to make the fungus fruit in artificial culture, was perfectly formed except that the ring was absent and thus it conformed with the macroscopic description of a sporophore of *C. tabescens*. It would seem that, in spite of Rhoads's careful descriptions, the two fungi are not easily distinguished. Heim & Jacques-Felix (1953) and Josserand (1930) have also expressed doubts on the validity of this distinction on other grounds.

DISCUSSION

The primary object of this investigation was to determine how far East African isolates of *A. mellea* differed among themselves and from others obtained from various parts of the world. No other work is known which covers isolates from as wide a geographical range as this study. Other workers on variation in *A. mellea* have studied isolates from a more restricted range of localities (Benton & Ehrlich, 1941; Hamada, 1940*a*, *b*; Lisi, 1940), although their observations were much more detailed than those described here. The observations on differences in morphology in relation to geographical origin of isolates are therefore thought to be new.

Particular attention was paid to the temperature-growth relationships of isolates of different origins, as this seemed likely to have a bearing on their pathogenic activity (Bliss, 1946). No relation has been found, possibly because differences in maximum soil temperatures in tropical and temperate regions are not very great.

I must express my gratitude for the generous response to requests for isolates of *A. mellea* that was made by mycologists from all over the world. Without this help the study could not have been started. I would also like to acknowledge the help of my assistant J. G. Kimani in the conduct of routine observations and experiments. Dr F. M. L. Sheffield has helped in reading and criticizing the manuscript, for which I am most grateful. Mr C. Spinage very kindly took the photographs, and Mr David Whitfield reproduced the diagrams.

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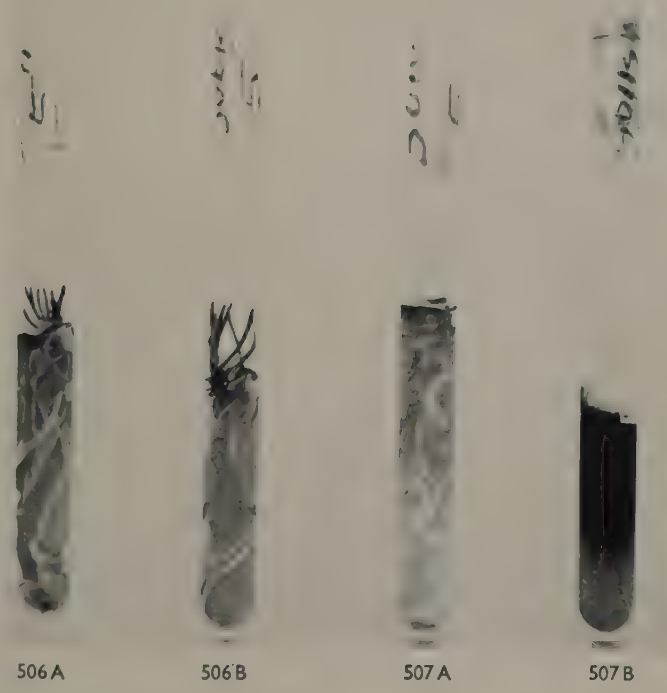
EXPLANATION OF PLATE 10

Fig. 1. Three types of vigorous rhizomorph growth. Left to right: types *c*, *b* and *a*. These cultures were removed from 6 × 1 in. tubes before photographing.

Fig. 2. Cultures showing aerial rhizomorphs (506 A, 506 B), normal rhizomorph production (507 A) and a non-rhizomorphic growth with copious pigment production (507 B). All in 5 × ½ in. tubes.

(*Accepted for publication* 24 *May* 1960)

(c)
(b)
(a)


506 A
506 B
507 A
507 B

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THE DEVELOPMENT OF COREMIA

I. *PENICILLIUM CLAVIFORME*

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(With Plate 11 and 3 Text-Figures)

The number and distribution of coremium initials and of mature coremia of *Penicillium claviforme* is markedly influenced by illumination and nutrition. Illumination also determines the size and form of coremia, the photosensitive period being early in the elongation of the coremium stalk, during the first 2 mm. of growth. At this stage also, and at no other, the coremia exhibit strong positive phototropism. Stalk elongation occurs by apical growth, and proceeds at a uniform rate until maximum height is reached. Following the cessation of growth, the differentiation of the head takes place, and sporulation follows. If the tips of growing coremia are amputated, regeneration occurs, and the tip briefly reverts to the photosensitive state characteristic of young coremia. A technique for growing excised coremia is described.

Penicillium claviforme Bainier and its striking coremia are briefly described by Raper & Thom (1949). The purpose of the present communication is to describe more fully the development of the coremia, their phototropism and other responses to light, and their ability to regenerate.

Experimental methods

Cultures were grown in a thermoregulated dark room, temperatures in the range 20–24° C. being employed. Illumination was provided at an intensity of about 100 foot-candles by means of Mazda 'daylight' tubes. These were operated by time-switches to give the desired illumination programme. Alternatively cultures were wrapped in opaque black paper to obtain dark conditions. The medium normally used was 3 % malt extract (Boots) solidified by means of 2 % Oxoid Kobe No. 1 agar, but various agar media based on the Czapek formula were also satisfactory. Petri dishes were commonly used, but where large coremia were required 5 cm. deep crystallizing dishes were employed. Investigations on coremium anatomy were made by teasing the hyphae apart with mounted needles, by clearing in 2 % KOH or lactophenol, or by means of sections obtained free-hand or with the freezing microtome.

Colony form

Illuminated cultures

Cultures grown in alternating periods of 12 hr. light and 12 hr. darkness, 16 hr. light and 8 hr. darkness, or in continuous illumination are all very similar in appearance. Coremia are produced throughout the colony in an apparently random manner, and are unbranched. They vary in height from about 4 mm. at the centre of the colony to about 20 mm. near the margin. These features are illustrated in Pl. 11, figs. 1 and 3.

Unilluminated cultures

Numerous very small coremia are produced near the point of inoculation. Further coremia are not then produced until growth ceases through staling, when large much-branched coremia develop at the margin of the colony (Pl. 11, figs. 2 and 4).

PHASES OF COREMIUM DEVELOPMENT

Production of initials

Cultures grown on malt agar in alternating periods of 12 hr. light and 12 hr. darkness show concentric zones of denser mycelium of darker colour. These zones consist of numerous roughly spherical coremium initials, about 0·5 mm. diam. and almost in contact with each other (Pl. 11, fig. 5). Each initial consists of a tangle of much-branched randomly orientated hyphae. These zones of coremium initials are produced at approximately 24 hr. intervals, and about 2 mm. behind the colony margin. Under conditions of continuous illumination or continuous darkness zoning is less precise.

Further development of these initials is determined by illumination and nutritional factors. On malt agar only a small fraction (e.g. 4 in a zone of 65) of the initials develop further, but on Czapek agar (Pl. 11, fig. 6) all the rather few initials develop into mature coremia.

Photosensitive phase of elongation

Elongation commences by the growth of a column of parallel unbranched hyphae from the initial. For the first 2 mm. of growth the coremium is photosensitive. Unilateral illumination produces a marked phototropic response, a reaction not obtained at any other stage in development of the fungus. Neither at this nor at any other stage of growth is there any evidence of geotropic sensitivity. Illumination in this phase also influences the final height and form of the coremium. Experiments with Wratten filters showed that the blue end of the visible spectrum was active both morphogenetically and phototropically; the red end was inactive.

The effect of illumination of young coremia on their ultimate form is illustrated in Text-fig. 1. Cultures having young coremia were transferred to darkness, and the heights of individual coremia at the time of removal noted. It will be seen that transfer to darkness prior to the beginning of elongation gives a fully 'dark', i.e. highly branched, coremium; transfer when a height of 2 mm. is attained gives a fully 'light', i.e. unbranched, coremium. Intermediate times of removal to darkness give intermediate results.

The effect of illumination on subsequent coremium height was ascertained by transferring to darkness cultures in which the coremia in the fifth zone from the centre were beginning to develop, and maintaining a similar set in light. The 'light' set of coremia subsequently attained an average height of 11·5 mm.; the 'dark' set reached 8·3 mm. This difference was significant at the 1 % level.

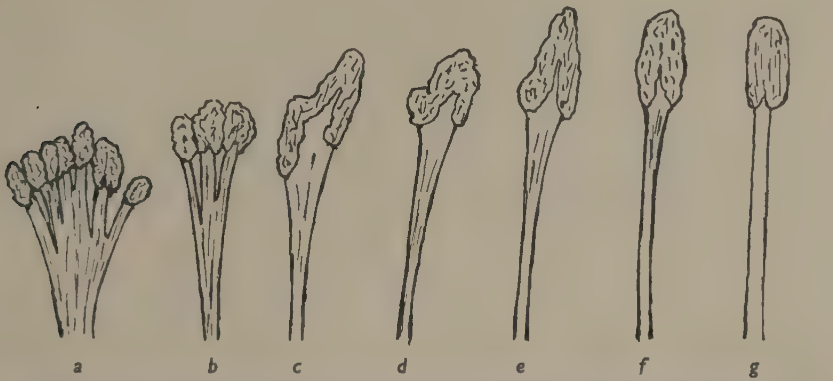


Fig. 1. Effect of illumination on coremium form. State of coremium at time of transfer to darkness was as follows: *a*, initial; *b*, commencement of elongation; *c*, 0·8 mm.; *d*, 1·2 mm.; *e*, 1·4 mm.; *f*, 1·6 mm.; *g*, 2·0 mm.

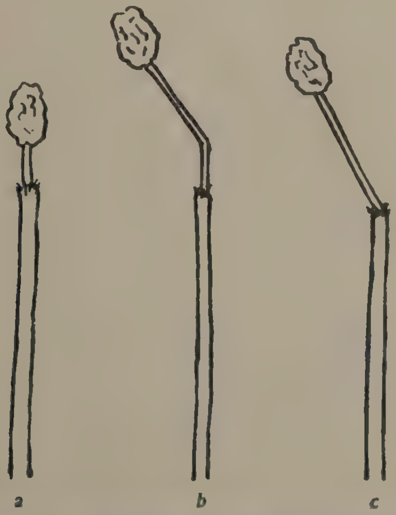


Fig. 2. Effect of illumination on coremium regeneration. Coremia transferred to unilateral illumination on new growth reaching *a*, > 1·5 mm.; *b*, 0·8–1·5 mm.; *c*, < 0·8 mm.

Light-insensitive phase of elongation

This phase is morphologically indistinguishable from the previous phase, but is characterized by loss of photosensitivity. Marking of coremia with Indian ink during this phase showed that growth was entirely apical. Measurements of coremium length at successive time intervals demonstrated the maintenance of a uniform growth rate of about

4 mm./day throughout the phase. Study of cultures in alternating periods of light and darkness showed that growth rate was unaffected by illumination. The stalk becomes pigmented during this phase at a distance of a few mm. behind the growing point.

At the microscopic level, this phase, like the last, is characterized by the growth of parallel, unbranched hyphae.

Differentiation of head

When elongation of the coremium ceases, lateral expansion of the upper part to form the head begins. At the microscopic level this is caused by the lateral growth and branching of hyphae. Finally, sporulation commences and the head becomes green.

REGENERATION

The removal of a coremium tip, during either the phase of elongation or of head differentiation, leads to the production of a new tip, and ultimately to formation of a head and to sporulation.

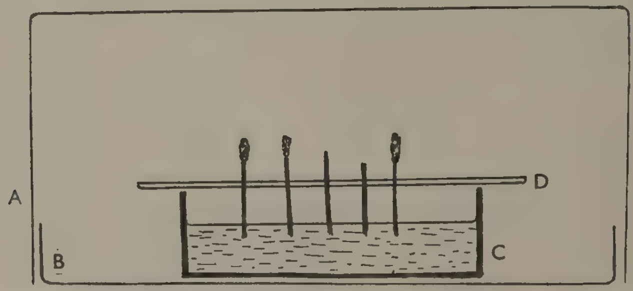
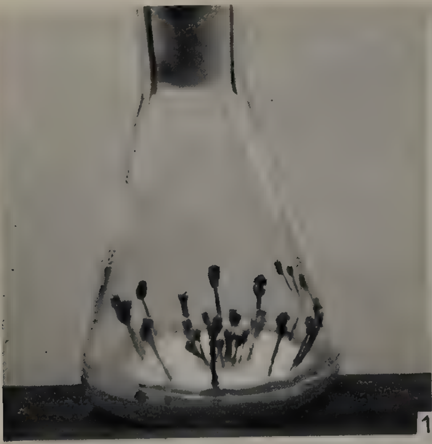


Fig. 3. Technique for growth of excised coremia. A, crystallizing dish; B, large Petri dish; C, small Petri dish containing distilled water or nutrient liquid; D, slide bearing coremia.

Following the removal of a coremium tip there is a period during which no observable regeneration occurs. This period is about 18 hr. with coremia still in the phase of elongation, but lasts about 30 hr. with those in which head differentiation is far advanced. The new tip is light-sensitive until it has reached a length of about 1·5 mm. Light affects ultimate length attained, illuminated coremia being longer, and, if the light is unilateral, causes phototropism (Text-fig. 2). Final lengths of the regenerated portion are usually of the order of 5–10 mm.

DEVELOPMENT OF EXCISED COREMIA

The possibility of studying the development of excised coremia was suggested by the work of Gruen (1959). The apparatus employed for providing aseptic conditions for studies on excised coremia is indicated in Text-fig. 3. Coremia are removed with forceps from the parent culture after cutting at the base with scissors. They are fixed to the edge of the slide by means of Lanolin; Vaseline was originally tried and found to be harmful.



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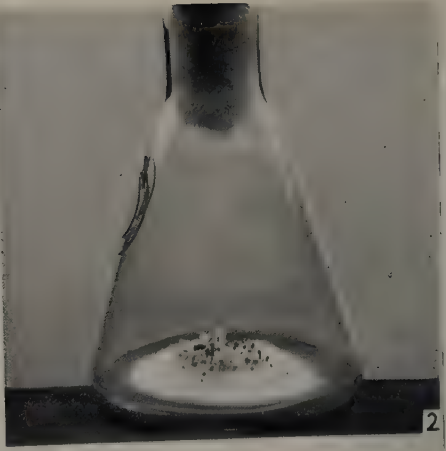

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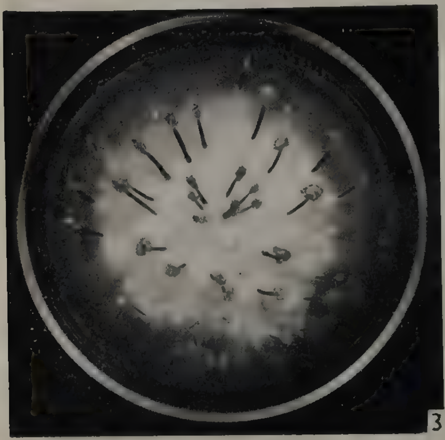

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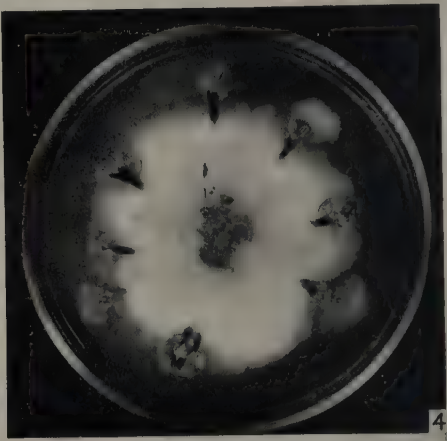

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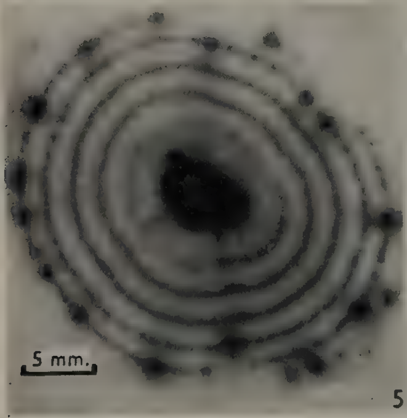

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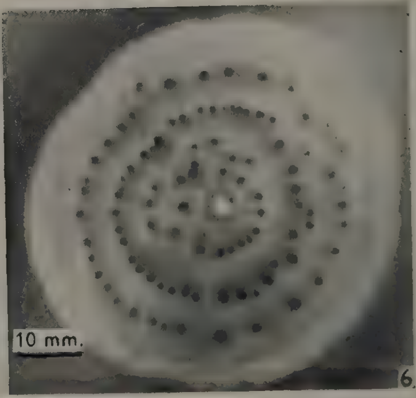

6

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If head differentiation has already commenced, the process is completed normally in excised coremia. If the excised coremia are still in the phase of elongation, elongation ceases and head differentiation commences. The heads produced are very short, but in other respects are normal. These results are not influenced by the use of 1 or 3 % malt extract in place of distilled water; this procedure leads, however, to the production of a mycelium from the coremium base in a few days.

Excised coremia are capable of regeneration following amputation of tips. The new tips are phototropic, and mature into small but otherwise normal coremia, the stalk formed being only about 1 mm. long.

We wish to thank Dr L. E. Hawker and Professor E. W. Yemm for helpful discussion, and Mr H. Joyner for photography.

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EXPLANATION OF PLATE 11

Figs. 1, 2. Two-week cultures on malt agar. Fig. 1, illuminated colony; Fig. 2, colony grown in darkness.

Figs. 3, 4. Three-week cultures on malt agar. Fig. 3, illuminated colony; Fig. 4, colony grown in darkness.

Fig. 5. Reverse of illuminated colony on malt agar. Note inoculum region, concentric zones of initials (tiny dots) with a few that have developed further (large dots).

Fig. 6. Reverse of illuminated colony on Czapek agar. All the few initials have developed into mature coremia. The greater distance between zones results from the high growth rate on this medium.

(*Accepted for publication* 24 *May* 1960)

PROCEEDINGS, 1960

MEETINGS

7 October 1960. Meeting held at Birkbeck College, London, W.C. 1, at 10.45 a.m. with the President, Mr E. C. Large, in the Chair.

THE CEREAL RUSTS

D. J. Griffiths (Aberystwyth). Breeding for resistance to crown rust in the oat crop.

In developing oat varieties capable of withstanding attacks of crown rust, the plant breeder has to concern himself with two important genetic aspects, (*a*) the pathogenicity spectrum of the existing race flora in the region, and (*b*) the isolation of resistance genes which, singly or together, will ensure a high degree of resistance to all the biologic forms of the pathogen when introduced into an adapted variety. Physiologic specialization in *Puccinia coronata avenae* is highly developed and the range in pathogenicity of some thirteen forms already found occurring naturally in Britain serves to indicate how the phenomenon of physiologic specialization has complicated the problem of breeding for crown rust resistance in oats.

The pathogenic reactions of these thirteen races were presented and an account given of the hexaploid, tetraploid and diploid genotypes of *Avena* manifesting resistance to all or several of these races. In addition, results of recent investigations with u.v.-irradiated uredospores of *P. coronata avenae* Race 96 were presented and discussed in relation to the mode of origin of new races.

D. A. Doling (Cambridge). West European yellow rust investigations.

In West European countries yellow rust (*Puccinia glumarum* Erikss. & Henn.) is the most destructive cereal rust, though variable meteorological conditions prevent the frequent recurrence of severe epidemics. Individual studies were made by Biffen (1907) on the inheritability of wheat resistance to the disease, and by Gassner & Straib (1930–39) on the physiologic specialization of the fungus, but severe epidemics in recent years, particularly in 1955, prompted an investigation on an international basis.

Following the first Yellow Rust Conference in 1956, sponsored by the Netherlands Grain Centre, a standard set of wheat varieties were sown as a 'nursery' at a large number of sites throughout Western Europe through the co-operation of many interested organizations. The regular observations made have been collected by the N.G.C. and are being analysed by Dr Zadoks. Some general observations can be made as a result of preliminary analysis.

During 1957–60 the general percentage infection, i.e. the yearly epidemic intensity, has been low, but severe epidemics occurred in Belgium and Holland in 1957 and in Holland and Northern Switzerland in 1958. The earliest records of yellow rust each year from each nursery support the view that the disease is endemic in most countries, the fungus perennating as mycelium in symptomless leaves. In Mediterranean countries, there is some evidence of spore migration from North Africa and possibly also from Kenya. Material collected from the nurseries and sent to Brunswick has been used for physiologic race identification. The most frequently identified races are to be found in all parts of the study area. In all, some nine races have been identified by Dr Fuchs, using the normal seedling reaction method. She has also made a detailed study of the usefulness and reliability of some of the differential hosts and proposed that some of them are no longer of value.

Races identified by seedling reactions in the glasshouse cannot always be easily related to field experience. Consequently an attempt to classify races on their adult plant reactions in the field is under investigation by Dr Zadoks.

These preliminary observations were presented at the second Yellow Rust Conference at Wageningen in June 1960.

R. C. F. Macer (Cambridge). Breeding for resistance to yellow rust in wheat.

Yellow rust (*Puccinia glumarum* = *P. striiformis*) is the most serious rust of wheat (*Triticum aestivum*) in Britain, although in recent years losses from the disease have been small because of the widespread cultivation of resistant varieties. It is, however, necessary to maintain and improve upon the existing level of resistance in new wheat varieties. The success of this breeding programme is dependent upon (1) efficient screening techniques to eliminate susceptible material, (2) the availability of additional sources of resistance to the north European physiologic races of *P. glumarum* and (3) an understanding of the genetics of resistance in the host.

Tests for resistance may be carried out in the glasshouse, upon seedling or adult plants, or in the field. In the glasshouse plants are inoculated by the 'dry spore dusting' method in which uredospores are blown from a 'Cyclone Duster' onto plants under large polythene humidity covers. Symptoms appear in 14–17 days and seven reaction types are recognized; three of these are non-sporulating (OO, ON, O) and four form a series of increasing sporulation and decreasing necrosis (I–IV) (Macer, 1960). The seedling test is used for (1) physiologic race determinations, (2) genetical investigations of the inheritance of major gene resistance, (3) selection of resistant plants from segregating populations, particularly in backcross programmes, and (4) screening for resistance to non-indigenous races of *P. glumarum*.

Resistance at the seedling stage is determined by major genes and is race-specific. Adult plant resistance is often less specific, may be polygenically inherited and can, at present, only be detected in the field. Field selection is, therefore, used to eliminate susceptible material from segregating populations after the establishment of a local epidemic in spreader beds of a susceptible variety.

The reactions of F2 populations and F2 plant progenies of crosses between seven wheat varieties have been examined after inoculation with four physiologic races of *P. glumarum* (2B, 5, 8 and 8B). The resistance of each of the resistant varieties at the seedling stage is controlled by a relatively simple major gene system, involving either a single gene pair or two independently effective gene pairs. Seven alleles conferring resistance have been identified, situated at four loci (*Yr1*–*Yr4*). Five of the alleles are inherited as dominants, one as a recessive and one, *Yr3* in the variety Minister, behaves as a dominant when tested with non-aggressive physiologic races and as a recessive when tested with aggressive physiologic races of *P. glumarum*. This suggests the possibility of a gene dosage effect (Lupton & Macer, 1960).

Genes for resistance are found in cultivated varieties of *Triticum aestivum*, in other *Triticum* species, i.e. *T. spelta album*, and in the allied genus *Aegilops* (*A. comosa* and *A. squarrosa*). Various combinations of the genes present in *T. aestivum* are being synthesized by hybridization into lines with an increased range of resistance; it is possible that these lines may also serve as additional differential host varieties. Progress has also been made in breeding programmes designed to combine resistance to yellow rust with resistance to mildew (*Erysiphe graminis* f.sp. *tritici*), to eyespot (*Cercosporella herpotrichoides*) and to loose smut (*Ustilago nuda* f.sp. *tritici*).

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R. S. Smith (Imperial College). Uredospore dissemination and infection.

Aerobiological studies were made over a wheat field infected with *Puccinia graminis*, using a Hirst spore trap and exposed vertical slides. Studies of uredospore liberation were also made in a miniature wind tunnel operated inside a constant environmental chamber. Maximum uredospore collections were obtained in the field when there was no surface wetness, when temperatures and radiation were at their highest, and when relative humidities were between 30 and 39%. The wind tunnel results generally substantiated these observations, apart from the effect of relative humidity which was in opposition to the results obtained in the field.

P. graminis uredospore gradients were followed from a point source and found to be hyperbolic and to follow closely the expected line for normal conditions of turbulence. Also the uredospore deposition gradient was found to be parallel to the disease intensification gradient.

L. Ogilvie (Bristol). The West European and British black rust investigations.

Epidemics of black rust of wheat (*Puccinia graminis tritici*) have been under close observation since 1947 at the N.A.A.S. Regional Centre at Bristol, since the worst effects, in epidemic years, have been felt in the south-west. Wild barberries (*Berberis vulgaris*), the alternate host of black rust, have been mapped in the south-west and to a lesser extent throughout the country, but there is no evidence that the wheat variety of the rust occurred on them during that period.

At the 4th International Congress of Plant Protection in Hamburg in 1957, a resolution was passed recommending an international investigation on black rust epidemics in Western Europe in view of the severe losses incurred. In January 1948, interested specialists met together in Bristol and a co-operative survey was organized. Dr I. G. Thorpe and the writer have organized field observations throughout the country with the help of N.A.A.S. officers, collaborating plant pathologists and others. Spore collections derived from the field and from widely scattered 'rust nurseries', planted with seed supplied by Dr J. C. Santiago, Portugal, have been used for the determination of races by him and by Drs Thorpe, Macer and Doling in England. Spore catching has been under the direction of Mr H. A. Hyde, National Museum of Wales, Cardiff, while special aerobiological techniques have been used by Dr J. M. Hirst, at Rothamsted. Preparation of air trajectories has been carried out by Mr W. H. Hogg, Meteorological Office, Bristol. By these means the progress of epidemics has been followed from 1958 onwards.

A report on the 1958 epidemic was given at the International Black Rust Symposium held at Versailles in October 1958. The present series of papers deals with salient points arising from the whole investigation and especially with the theory of airborne transport of uredospores from the south. These epidemics, ranging from mild to severe, occur in England practically every year, extending all over the south and adjacent islands and northwards to the midlands, and possibly to Ireland.

The West European investigations have shown that black rust overwinters in the uredospore stage on wheat and grasses in North Africa and Southern Iberia and spreads northwards throughout spring and summer every year. The British observations show that our own epidemics originate from epidemics to the south and that the rust problem is in fact the same as that of West Europe generally. Any measures, such as the introduction of varieties resistant to common West European races, which will greatly reduce the amount of black rust in France and Iberia, are likely, therefore, also to benefit England.

Severity of black rust epidemics in England depends on susceptible varieties of wheat (all varieties given here are susceptible to the common West European races of black rust), lateness of the wheat crops, transport of spore clouds arising from epidemics to the south by one or more favourable wind trajectories in late June and early July, and warm and humid weather at the time of infection and for several weeks later.

I. G. Thorpe (Bristol). Recent work at Bristol on black rust epidemics.

Black stem rust of wheat (*Puccinia graminis tritici*) caused serious damage in south-west England in 1947, 1950, 1951, 1952 and 1955, when severe epidemics developed. The epidemiology of the disease has been studied at the Bristol headquarters of the N.A.A.S. About forty widely scattered bushes of the common barberry (*Berberis vulgaris*) have been found in south-west England and these frequently carry aecidia of *Puccinia graminis*. Inoculation studies, spore measurements and observations on species of *Gramineae* infected nearby indicate that the bushes are frequently infected by the varieties *avenae* and *secalis*, but the variety *tritici* has never been observed on barberries in south-west England.

The simultaneous appearance of the disease in southern England suggests a massive inoculum over a wide area. Uredospore deposits on traps on the Bishop Rock Lighthouse, and at Cardiff (information supplied by Mr H. A. Hyde and Mrs K. F. Adams), can be correlated with the movement into south-west England of air from France and occasionally from Spain and Portugal. Air trajectories for the May to July period have been tracked by Mr W. H. Hogg. Peak periods of spore catches usually coincide with the arrival in England of trajectories from the south.

Races of *P. graminis tritici* have been identified over the past 5 years at the Plant Breeding Institute, Cambridge (Dr R. C. F. Macer), at the National Institute of Agricultural Botany, Cambridge (Dr D. A. Doling), and at Bristol (author), and during the past 3 years at Elvas, Portugal (Dr J. C. Santiago). There is a close similarity both in the identity and proportion of races found in England, in France and in the Iberian Peninsula. The evidence suggests that the disease originates from uredospores brought in from countries to the south, and that severe epidemics may follow early heavy spore deposits in years with high summer temperatures.

W. H. Hogg (Bristol). Meteorology in relation to recent black rust epidemics.

Epidemics of black rust are dependent on meteorological factors in that the spores may be transported over long distances and are affected by the weather of the area in which they are deposited. An estimate of the possibility of transport to or from particular areas may be made by tracking trajectories. In the absence of a close network of wind observations, particularly over the sea, trajectories may be tracked by using pressure charts for appropriate levels. These are known as geostrophic trajectories and they are subject to error from two main causes: (*a*) the difference between the true wind and the geostrophic wind, (*b*) errors in the measurement of the geostrophic wind.

Adapting values given by Murray (1954) and Durst & Davis (1957), the errors from 6-hourly surface charts and 12-hourly charts at 700 and 500 mb. (approximately 3000 and 5600 m. above sea level) are shown to be as follows.

Standard vector errors of position after stated periods
(nautical miles)

	6 hr.	12 hr.	24 hr.	2 days	3 days	4 days	5 days
Surface	42	59	84	119	145	168	188
700 mb.	—	120	170	240	294	339	369
500 mb.	—	168	237	336	411	475	531

Starting from Plymouth, trajectories have been tracked backwards in time for maximum periods of 5 days. This has been done for each day in May, June and July for the years 1947–60, and for the levels of approximately 600, 3000 and 5600 m., based respectively on the surface isobars and on the contours of the 700 and 500 mb. surfaces.

The main interest in the work on black rust has been in trajectories from the Iberian Peninsula and from France. These have been plotted in calendar form for use in comparison with the biological data. It is clear that there must be difficulty in distinguishing between possible tracks from the Iberian Peninsula and those from France, as most of those which pass over Spain and Portugal also pass over some part of France, even if only over the Brittany Peninsula. The main feature on synoptic charts and contour charts associated with trajectories from the Iberian Peninsula is the presence of a low or a trough over, or somewhat to the west of, the Bay of Biscay.

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H. A. Hyde & K. F. Adams (Cardiff). Spore trapping in relation to recent black rust epidemics.

The results of the pollen and spore census centred on Cardiff from 1942 onwards have shown that the great bulk of spores trapped in Great Britain had originated within a few miles. But in almost every year, a few pollen grains or spores have been caught at times when they could not have been liberated inside the British Isles; in particular, a larger or smaller influx of pine pollen grains reaches Great Britain during the spring of every year, when pine trees are flowering in south-western Europe but not in Great Britain. Similarly, small numbers of spores of *Puccinia graminis* have been caught between 1 May and 15 July every year from 1947 onwards (when these particular observations began), except 1951. Since (as is understood from the plant pathologists) the earliest of these spores to be caught in each year antedated any possible outbreaks of black rust in Great Britain, it is concluded that these out-of-season rust spores must have been blown in from the continent of Europe or possibly even from across the Mediterranean.

J. M. Hirst (Rothamsted). The aerobiology of *Puccinia graminis* uredospores.

The geometric mean diurnal periodicity of *Puccinia graminis* uredospore concentrations, calculated from the catches by Hirst spore traps at ½ m. above ground in infected spring wheat, shows an afternoon maximum (see Hirst, 1953), but the highest concentrations occur erratically and often at night. In 1959 and 1960, more than half of the brief peak concentrations coincided with the start or an intensification of rain. The occurrence of up to 11,000 uredospores per cu.m. air, at night, raised questions about the method of spore liberation. Tests in a very small wind tunnel, where spore liberation could be measured and observed microscopically, had suggested that catches increased logarithmically with wind speed but that exposure to wide differences in humidity was not a major factor controlling liberation, although dry air may have favoured it slightly. This agrees with the hypothesis that uredospores are abscissed some time before liberation and are presented for dispersal as a floccose powder of detached spores. Individual spores are probably difficult to wet and are not sticky. They may tend to adhere to one another because of the hooked spines which cover their surface, and which are readily visible on electron micrographs of carbon replicas of uredospores.

The evidence suggested that the erratic peak concentration resulted more from the collision of raindrops with spore-bearing surfaces than from changes in the ambient air temperature, humidity or velocity. This hypothesis was tested by allowing water drops (3·4 mm. diam.) to fall with terminal velocity onto infected straws oriented vertically or horizontally within a glass cylinder (6 in. diam. and 8 in. tall). Microscope slides, coated with Naphthol Green B in gelatine (Gregory, Guthrie & Bunce, 1959), were fixed on the inner wall of the chamber and caught many uredospores dispersed by splashing, despite the reputed difficulty of wetting them. Although splash dispersal may be important in spreading the disease locally it was not thought likely to explain high concentrations of air-borne spores. A small impactor (Gregory, 1954) was installed within the chamber, with its orifice directed tangentially, to sample the air before, during and after exposing specimens to 9 drops (3·4 mm. diam.) per min. Table 1 summarizes the results. To determine the proportion of spores caught dry or in droplets, the trap slide exposed during dropping was first coated with Naphthol Green B and then half over-coated with 'Vaseline'. The 'Vaseline' part caught all spores, whether dry or in droplets, the area where Naphthol Green was exposed caught few dry spores but showed the number of droplets caught and how many spores these contained (Table 1). Thus, most uredospores in the air were dry when caught and the number released, from samples of approximately 12–24 in. of straws with a moderately heavy infection, is probably large enough to explain the brief but large increases in spore concentration within crops at the start of rain. The tests so far have not shown whether raindrop collision releases dry spores simply by mechanical shock or operates as a more complex cause.

Table 1. *Analysis of catches* within glass chamber before, during and after dispersal of* Puccinia graminis *uredospores by raindrop collision*†

Test No.‡	Estimated total catch				
		During dropping			
	Before dropping uredospores	Uredospores	Splash droplets	Uredospores in splash droplets	After dropping uredospores
Sp. 1	144	13,420	—	—	2
Sp. 2	23	13,550	524	0	5
Sp. 3	0	9,430	1,975	252	0
Sp. 14/2	0	311	96	0	0
Sp. 15	0	274	46	0	2
Sp. 16	0	128	82	0	2

* Total catch was estimated by scanning 44 % of the area beneath the trap orifice on all slides except those 'during dropping' in Sp. 2 and later tests, when only 22 % of the area was scanned.

† Nine water drops (3·4 mm. diam.) falling per min. at their terminal velocity.

‡ Air sampled at 10 l./min. in all tests, for 10 min. per slide in Sp. 1 and 5 min. per slide in all subsequent tests. Target straws were held vertically in Sp. 2 but horizontally in all other tests. Lightly infected late tillers had to be used for Sp. 14/2, Sp. 15 and Sp. 16.

The association between geostrophic air trajectories and catches of uredospores in ground spore traps, established in preceding papers in this Symposium, suggests that immigrant uredospores may infect wheat in England. Attempts have been made to sample air masses of southern origin by using a special suction trap installed in aircraft of the Royal Air Force Meteorological Research Flight. Triplicate samples of 1–2 min. duration (30–80 l. of air) were taken at 2000 ft. above sea level at intervals along a traverse across the air trajectory, and during an ascent to 10,000 ft. at the mid-point of the traverse. Uredospores were caught over the English Channel in 1957, 1958 and 1959 on days when ground traps suggest that immigrant uredospores were important. The highest estimated concentration of *P. graminis* uredospores was 180 per cu.m. air at 2000 ft. on 9 July, 1959. Sometimes they were most common at between 3000 and 5000 ft. above sea level, whereas under the same unstable conditions the concentration of most other spore types declined logarithmically with increasing height.

Acknowledgement is made to the Staff of the Meteorological Research Flight and to Mr M. V. Carter, Mr O. J. Stedman and Miss Maureen Thomson for their collaboration.

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MISS GULIELMA LISTER CENTENARY

On 28 October 1960 at 3 p.m., there was a joint meeting with the Linnean Society in the new Lecture Theatre of the British Museum (Natural History), South Kensington, to celebrate the centenary of Miss Lister's birth and of her long association with the two Societies and the Museum. The meeting, which was presided over by the Presidents of the two Societies, Prof. C. F. A. Pantin and Mr E. C. Large, began with a series of tributes to Miss Lister. Lord Cranbrook spoke on 'Miss Lister and the Lister family'; Dr J. Ramsbottom on 'Miss Lister and the British Museum and the Linnean Society'; Dr G. C. Ainsworth on 'Miss Lister and the British Mycological Society' and Dr E. M. Delf on 'Miss Lister and the Botanical Research Fund'.

This was followed by tea and the inspection of an exhibition of material connected with Miss Lister, arranged in the adjacent Exhibition Room. The exhibits included examples of Miss Lister's drawings of myxomycetes, fungi, flowers, and birds, sheets from Miss Lister's herbarium of flowering plants (which she gave to Bedford College), specimens of myxomycetes, examples of the myxomycete notebooks (see these *Transactions*, **35**, 188, 1935), the deeds of the Botanical Research Fund, and, among more personal relics, the pair of vases presented to Miss Lister by the Emperor of Japan.

(For further details of Miss Lister, see G. C. AINSWORTH & FRANCES L. BALFOUR-BROWNE, *Nature, Lond.*, **188**, 362–363, 1960.)

REVIEWS

Herb. I.M.I. Handbook. (Commonwealth Mycological Institute, Kew, Surrey, 1960). Pp. 103, 6 plates, 8 text-figures. Price: 12*s.* 6*d.*

This handbook, with a mysterious title of abbreviations understandable only to the initiated, contains many new revelations even for the experienced mycologist. The book is dedicated to Mr Mason, who has devoted his life to the classification of micro-fungi and is the central pillar around which the herbarium was built up. This publication is unique in the wide range of subjects it covers; the contributions by different authors, based on their own experience, give it a very lively style. I would recommend that Mr Deighton's chapter, on how to describe a fungus, be studied by every mycologist before he decides to put his name behind what he would like to consider a sp.n.!

Dr Wiltshire reviews the history of the Institute, discusses the many activities of its staff, past and present, and summarizes the backgrounds of the invaluable publications, the *Review of Applied Mycology*, the handbooks, *Mycological Papers*, the *Index of Fungi*, the *Dictionary of the Fungi*, and others. The short chapters on the 'Index of Fungi' (Mr Deighton), 'Bibliography of Systematic Mycology' (Miss Waterhouse), 'Review of Applied Mycology' (Mr Martyn) and 'Review of Medical and Veterinary Mycology' (Dr Ainsworth) give us an insight into the work involved in their preparation.

Generally if one wants to learn about the practices and techniques employed at an institute, one must go and inquire. Reading this book the 'visitor' gets a carefully prepared guided tour through the different departments of the Commonwealth Mycological Institute. Dr Ellis takes us to the Herbarium where we are given a clear definition of specimen and species and much valuable advice about collecting and preserving specimens. Methods for making slide cultures and dried Petri dish cultures are described clearly and in detail so that they can be worked out even by a beginning mycologist.

Major Dade shows us the collection of living fungi, at first pitying the poor moulds because of all the horrors to which we expose them in pure culture, so widely different from their natural habitat. Then he gives us advice on methods ameliorating these bad conditions. Culture media, glassware, conditions of light and temperature, storage under oil, control of our enemy the mite—all are discussed. We learn methods, worked out in many years of experimenting, of making and sealing microscopical preparations. We are advised to keep detailed records of new accessions, giving each a number which will appear in all records of this specimen in the herbarium as well as in the culture collection. Whereas in the herbarium we learned a method to make cultures on slides, here we learn still other methods: cultures on cover glasses and in micro-jars.

Miss Waterhouse gives us some special tricks for the treatment of important pathogenic Phycomycetes, which often give trouble because of their reluctance to produce zoospores or oogonia in culture.

A chapter by Dr Hayward on 'Bacteria and the Herbarium' introduces to us a new branch of the C.M.I. Helpful advice is given to plant pathologists on how to isolate the causal organisms and how to dispatch the material to the C.M.I. for examination. However, I am afraid the bacteria will make poor herbarium specimens!

A list of staff members and an impressive list of publications issued by the Institute complete this stimulating handbook. Although it is by no means intended to be an introduction to mycology, beginners as well as advanced mycologists will find much to their profit. They will be fascinated by the tour through the C.M.I. and by the richness of advice based on experience given in these hundred pages.

A. L. VAN BEVERWIJK

The Ecology of Soil Fungi. An International Symposium. Edited by D. Parkinson and J. S. Waid. (Liverpool University Press, 1960.) Pp. xi + 324, numerous text-figures. Price: 42*s*. 6*d*.

A 2-year lapse between the holding of the symposium and the appearance of this volume of the proceedings in no way detracts from the value of the work, as sometimes happens with more ephemeral material. Of the ninety workers from thirteen countries attending the symposium thirty-five, of ten nationalities, contribute papers to the volume, and others are represented in the accounts of the discussions. Each of the six sessions was begun by a review of the topic under consideration given by an authority in that field, was continued with three to five other papers, and was rounded off with an open discussion. Sessions on methods for isolating fungi from and estimating their activity in soil, the growth of fungi in soil, antagonisms in soil, dynamic equilibria in soil populations, decomposition of organic matter in soil, and the physiology of soil fungi were introduced respectively by J. H. Warcup, J. S. Waid, P. W. Brian, N. A. Burges, C. G. C. Chesters and J. L. Harley. The approach of most of the other contributors was in keeping with the broad review treatment of these leading papers, so that in general the title of the whole symposium was kept in view and the detailed data were related to wider aspects of the subject than those immediately studied. Only some half-dozen of the twenty-eight papers are without any serious attempt at reference to broader ecological ideas.

Considerably more space is given to currently favourite topics like growth in soil and antagonisms in soil than to the problems of dynamic equilibrium in soil and physiology in soil. Nevertheless, sufficient attention is given to these last two to show that here are important subjects for study that will probably increase in importance in the future. The titles of the papers show an overall content that is narrower than one might have thought desirable, the bulk of the work described being 'non-applied'. Apart from a small number of references to rhizosphere problems, the synecological relationships between fungi and living higher plants are neglected. The almost simultaneous holding in the U.S. of a congress on these aspects must have attracted a number of workers who otherwise might have attended this present symposium and remedied the balance in this respect. Nevertheless, whether work is 'pure' or 'applied', or the plant material living or dead, the ecological principles with respect to the fungus are similar, and the narrower scope of the volume does not necessarily reflect a smaller value in conceptual content.

It has often been suggested that the main value of international scholarly gatherings of this sort lies in the unrecorded informal discussion that takes place outside the lecture hall, a view that is supported by the fact that many published proceedings of such meetings give the impression of data scraped together to make papers, to qualify their authors for assistance in attending. This criticism is not appropriate to this symposium; the papers here are of high quality. The informal discussions and the discussions at the end of each session did, nevertheless, form an important part of the proceedings. This, unfortunately, is not well brought out in the printed version of the recorded discussions, where it is often difficult to know to what particular point an item of discussion refers. Too much of the discussion in fact seems to float unrelated to what has gone before. This is probably not so much the fault of the discussion as of the method of direct transcription of the spoken comment. When composing a statement to be spoken, one is naturally less careful of subtlety of construction, relying on intonation (and gesture) for exact weight of meaning. A comment of this sort put without modification into print loses much. Despite this criticism the discussions contain a considerable number of important points made and relationships pointed out, so that their inclusion here adds to the importance of the work.

In the introduction to the volume, Professor Burges points out that the present time is a period of transition in the study of soil fungi and that the next few years will see big changes in the subject. One feels that some of these changes are foreshadowed in some of the articles in this work, the general tone of which is enthusiastically forward-looking, rather than rooted in the past. For this reason one would not lightly recommend it to the newcomer to soil mycology, who could easily become lost in it and miss much that it contains. But on the shelf of the worker already familiar with basic ideas, this is a volume that will not gather much dust.

D. PARK

Practical Microscopy. By C. L. Duddington. (London: Sir Isaac Pitman and Sons, Ltd., 1960.) Pp. vi + 237, 81 text-figures, frontispiece, and 3 plates. Price: 30*s.*

A good, up-to-date guide to the microscope, written for professional and amateur users of the instrument, long overdue, is now provided by Dr Duddington. He is a biologist who understands the theory of the microscope, and the result is a commonsense treatment in place of the usual academic one of physicist authors. There are only half-a-dozen equations, simple and useful, in the first part of the book, which deals with the instrument itself and its use in the small space of 152 pages. This feat of compression has been achieved by the author's gift of economy of words combined with great clarity; the information given is comprehensive and adequate for its intended purpose.

An excellent introductory chapter on simple optical theory is followed by a description of modern stands and lenses, and useful notes on buying a microscope. The supremely important subject of illumination, commonly little understood by those who use the microscope as a tool, is very properly given a chapter to itself, as is also the process of setting-up and using the instrument. Methods of drawing, micrometry, counts, and projection are briefly, but suitably, dealt with. A chapter on photomicrography is sound so far as it goes; the use of 35 mm. film, to which many of us are driven by the high cost of other negative material, is not mentioned. This first part of the book deserves high praise, and well repays the price.

Part II deals with microtechnique, and here again the author has packed a great deal into 79 pages, but the field covered is so great that the information must be regarded only as a useful general survey. It appears to indicate the limited methods in use in a teaching establishment up to graduate level. It could have been a useful directory if the bibliography had included references to specialized technical handbooks on the many different classes of biological material. Only a small space is devoted to fungi, and mycologists will find nothing new. The formula given for lactophenol is incorrect, and trypan blue, now replacing cotton blue, is not mentioned. Perhaps such criticism is facile, and it should not detract from the over-all value of this useful book. H. A. DADE

Illustrated Genera of Imperfect Fungi. By H. L. Barnett. (Minneapolis: Burgess Publishing Company, 2nd Ed., 1960.) Pp. iii + 462 figures. Price: U.S. $4.50.

This second edition is a great improvement on the first (these *Trans.* **38**, 480, 1955). The drawings are to a slightly smaller scale, there are more of them to a page and the arrangement is much neater: 462 genera are illustrated and briefly described instead of 302 and, as before, a key is provided. The usefulness of the book has been greatly enhanced by the bringing together of references at the end instead of having them repeated many times throughout the text. This edition should be on the shelves of everyone who has to identify Fungi Imperfecti.

I have two general criticisms to make. First, too little attention is paid to the precise way in which spores are formed and the drawings are sometimes misleading. An example of this is on pp. 64–5 where the sporogenous cells of *Scopulariopsis* are shown as phialides when they are, in fact, short annellophores. The fungus drawn on p. 59 to illustrate the genus *Fusidium* looks much more like a species of *Paecilomyces.* The figure illustrating the genus *Chloridium* on p. 97 is highly reminiscent of a *Tritirachium*, but as Dr S. J. Hughes has shown, *Chloridium* provides an early generic name for fungi which have been included in such genera as *Bisporomyces.* Secondly, and this is a more serious matter, generic names which modern research has shown should be discarded are being perpetuated. Examples of this include: *Acrotheca* (p. 99), the type species of which is a *Ramularia*; *Acrostalagmus* (p. 55), a synonym of *Verticillium*; *Hormiscium* (p. 85), a synonym of *Torula*; and *Scolicotrichum* (p. 105) which has been shown to be based on plant hairs associated with *Diplodia* spores. M. B. ELLIS

Amanites du sud-ouest de la France. By A. G. Parrot. (Biarritz: Centre d'Études et de Recherches scientifiques, 1960.) Pp. 173, 47 figures, paper cover. No Price.

This monograph is based on specimens seen and studied by the author, descriptions being given of twenty-three species and forty-five infraspecific taxa within these species. They are grouped according to the Gilbertian genera, segregates of *Amanita*, viz. *Amanitaria*, *Amanitina*, *Amidella*, *Amplariella*, *Aspidella*, and also *Amanitopsis*, though except in a few instances these names are not used in the binomials in the text, but are used in the captions to the figures. The main part of the work is preceded by a key to the genera, an historical review of previous work on this group in the area and a four-page bibliography. It concludes with a section on spores (with figs.) and one on distribution and ecology. Seven of the species described and all but a few of the infraspecific taxa are not in the British List.

The disadvantages of the book are the absence of coloured plates, the incorrect author citations, and the indiscriminate use of var. and form for one taxon. The book is well produced on good paper and the descriptions are reasonably full.

G. M. WATERHOUSE

Champignons de France. By A. Maublanc. 5th edition revised by G. Viennot-Bourgin. (Paris: Paul Lechevalier, 1959.) Vol. 1, pp. 308, 59 text-figures; Vol. 2, pp. 283, 224 coloured plates. Price: 5500 francs.

In this edition all the plates and accompanying descriptions are in volume 2. Volume 1 comprises the general introduction with keys to the genera. About a dozen plates have been replaced by new ones, not always with happy results; I found the illustration of *Tricholoma rutilans* far less satisfactory than the original one. A few specific names and rather more generic names have been changed, and some of the new ones might be difficult to justify; *Boletus scaber* has become *Trachypus carpini*. *Lactarius pyrogallus* is still so named but looks (to me) more like *L. circellatus*. The numbering of the plates is unchanged. My impression is that, in some cases at least, the quality of colour reproduction falls short of that of the earlier editions; *Tricholoma saponaceum* has undergone a remarkable change of colour—perhaps not very serious in such a variable species.

There is still no published work to replace these two volumes for the purpose for which Professor Maublanc wrote them, and even with the new value of the franc they are still a good buy. The photograph of this eminent mycologist, which forms the frontispiece of volume 1, will give great pleasure to all.

F. B. HORA

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INSTRUCTIONS TO AUTHORS

A revised and amplified version of our *Instructions to Authors* will appear on p. ii of Cover in future June and December numbers, alternating in this position with the *List of Officers and Council*, which will henceforth be printed in the March and September numbers only.

THE EDITORS

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